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Hot-spot application of biocontrol agents to replace pesticides in large scale commercial rose farms in Kenya

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Abstract Rose (*Rosa hybrida* L.) is the most important ornamental crop in Kenya, with huge investments in pest management. We provide the first full-scale, replicated experiment comparing cost and yield of conventional two-spotted spider mite (*Tetranychus urticae* Koch) control with hot-spot applications of the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae) in large commercial rose greenhouses. Hot-spot treatments replaced acaricides except at high infestations and the two treatments were applied in seven greenhouses each. With the conventional treatment, acaricides were applied when *T. urticae* populations exceeded 250 motile individuals per plant based on scouting. Treatments with acaricides and *P. persimilis* were guided by weekly scouting and hot-spot treated greenhouses with infestations exceeding 1000 individuals m⁻² (calculated as average mites/leaflet × average leaflets per plant)

were first blanket-treated with an acaricide to decrease infestations. Roses subjected to the hot-spot treatment had significantly lower *T. urticae* infestations compared with conventionally treated roses. In addition, significantly fewer high spider mite infestations were recorded in roses with the hot-spot treatment. The cost of pest management was significantly lower in the hot-spot-treated greenhouses than in the conventional treatment. However, there was no significant difference in the number of harvested stems from the two treatments. It can therefore be concluded that acaricides can be replaced by *P. persimilis* hot-spot treatments in commercial cut rose production, effectively reducing pest management costs with no loss in crop yield.

Keywords Acari · Phytoseiidae · Cost-benefit · Rose · *Phytoseiulus persimilis* · *Tetranychus urticae*

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Introduction

Kenya is a major producer and exporter of horticultural commodities, flowers being the highest in volume and value of the exported horticultural products. The cut flowers industry is among the fastest growing sectors of the Kenyan economy, with revenues of approximately \$US 0.5 billion in foreign exchange (HCDA 2009) based on an exchange rate of 1 \$US = 80 KES. Rose (*Rosa hybrida* L.) is the most

important cut flower, amounting to 77 % of the flower volume and 62 % of the flower value in 2009 (HCDA 2009). A major proportion of rose production in Kenya takes place in large commercial greenhouses and in such greenhouses, pest infestations develop quickly, and the costs of pest control constrain rose production. The twospotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a highly important rose pest, often constituting the main expense in pest management budgets (Zhang 2003; van de Vrie 1985). The control of spider mites has been based mainly on the use of acaricides, resulting in pesticide resistance and accumulation of pesticide residues on the harvested products (Attia et al. 2013; Khajehali et al. 2011; Escudero and Ferragut 2005). In addition, acaricides are known to be highly toxic to farm workers, non-target organisms and the environment (Znaor et al. 2005). The current demand for good agricultural practices by trading partners and consumers has influenced the choice of pesticides in crop production and many growers have opted for the use of biocontrol agents such as *Phytoseiulus persimilis* Anthias-Henriot (Acari: Phytoseiidae), which effectively reduces *T. urticae* populations in floricultural crops (Holt et al. 2007; Casey et al. 2007; Opit et al. 2004; Nicetic et al. 2001; De Vis and Barrera 1999) as well as other crops such as vegetables (Zhang 2003). *P. persimilis* is a specialized spider mite predator and feeds on all life stages of *T. urticae* and its populations build up rapidly when food is plenty and climatic conditions are favorable. However, it is difficult to establish and maintain when prey densities are low, so it is exclusively used for inundative biological control (Casey et al. 2007). Low pest tolerance by growers can therefore make successful introduction of biological control with *P. persimilis* in commercial production difficult (De Vis and Barrera, 1999). In Kenya, utilization of this predatory mite within commercial flower companies was initiated by Dudutech, a commercial producer of biocontrol agents since the late 1990s. Growers in Kenya usually release *P. persimilis* uniformly, based on spider mite density (Jacobson 1993). *Tetranychus urticae* moves slowly, but is easily dispersed by air, which leads to a patchy distribution of *T. urticae* in the crop (El-Laithy and Sawsan 2005), and areas of aggregation in the greenhouse become a source of its spread. Targeting these areas of aggregation, or hot-spots, could limit spread and build-up of *T. urticae* (Alatawi et al. 2011),

thus effectively reducing exposure of workers and environment to acaricides and potentially reducing costs of spider mite control. In this study, we compare two strategies for spider mite control in commercial rose production with respect to mite control, cost of pest management and yield: a) uniform application of acaricides, i.e. the conventional treatment, and b) hot-spot applications of *P. persimilis*, replacing acaricides except at high infestations.

Materials and methods

The study was conducted in commercial rose greenhouses on Kingfisher Farm at the shores of Lake Naivasha, Rift Valley province, Kenya (0°47'60N, 36°21'0E), which lies at an altitude of 1800–2000 m above sea level. Within the farm, 14 plastic covered greenhouses were randomly selected, all with roses cultivated on raised beds in a hydroponic cultivation system. These greenhouses were part of the farm's commercial greenhouses and they continued to provide commercial flowers during the 30 weeks of trials, weeks 1–30, in 2011. In 2011, the Kingfisher farm weather station recorded a mean monthly temperature of 19 ± 1.1 °C, an average monthly minimum temperature of 11 °C, an average monthly maximum of 27 °C and an annual precipitation of 814 mm (monthly range 3–133 mm). All greenhouses were within a distance of 500 m from each other, hence had similar climatic conditions and in all greenhouses the crop was already well established before the start of the experiment with a density of eight plants m^{-2} . The 14 selected greenhouses were from 4100 to 16,820 m^2 large and had housed seven different rose varieties, each occupying two greenhouses (Table 1), and for each rose variety one greenhouse was used as a control (i.e. conventional treatment) and the other was used for the hot-spot treatment, and none of the varieties had known resistance to *T. urticae* or were known to have negative effects on *P. persimilis*. Treatments against *T. urticae* included the use of *P. persimilis* obtained from a commercial rearing unit at Dudutech, a subsidiary of Finlay Horticulture (K) LTD, and acaricides (Table 2). Dudutech is located within the Kingfisher farm where the trial was carried out, and produces *P. persimilis* commercially, so predators could be delivered one day after harvesting.

Table 2 Acaricides applied in the greenhouses

Active ingredient	Trade name	Rate (ml l ⁻¹)	Management option
1 % Milbemectin	Milbeknock 1 %EC	50	Both
Abamectin	Dynamec 1.8 EC	50	Both
Abamectin	Zoro TM 18 EC	50	Both
Abamectin 2.0 g l ⁻¹	Abamite 2.0Ec	30	Both
Alpha cypermethrin	Fastac 10 EC	30	Conventional
Bifenazate	Floramite 240 SC	40	Hotspot
Chlorfenapyr	Secure 36 SC	50	Conventional
Clofentezene	Apollo 50 SC	30	Hotspot
Clofentezene 500 g l ⁻¹	Efentezine 50SC	60	Hotspot
Etoxazole	Baroque 10SL	50	Hotspot
Hexythiazox	Nissorun 10 EC	50	Hotspot
Hexythiazox 100 g l ⁻¹	Hexygon 10 %Ec	50	Both
Profenofos 400 g l ⁻¹ + Cypermethrin 40 g l ⁻¹	Polytrin P 440Ec	60	Conventional
Pyridaben	Dynomite 15 EC	90	Conventional
Pyridaben 200 g l ⁻¹	Pyrimite 200 EC	80	Conventional
Spiromesifen	Oberon SC 240	50	Conventional
Tebufenpyrad	Oscar 200 EC	30	Conventional

The column management option refers to whether the acaricide quoted was used in hot-spot treated greenhouses, in controls (conventional) or in both treatments

Phytoseiulus persimilis was applied as a hot-spot treatment using 200 ml bottles each containing 2000 predators, with vermiculite as a carrier material to enable uniform distribution of the predators, and the bottle was rotated while sprinkling the contents on the crop, and before application, the number of plants each bottle would treat was calculated in order to reach a *P. persimilis*:*T. urticae* ratio of 1:10. Acaricides were applied as conventional treatment uniformly within the greenhouse and to safeguard flower production acaricides were allowed to be sprayed in every greenhouse irrespective of the treatment when *T. urticae* populations attained the acaricide action threshold level (i.e., 250 motile *T. urticae* per plant on average based on scouting data), as such an agreement was necessary for greenhouse managers to allow the conversion to hot-spot treatment in these greenhouses. Scouting and spraying followed the normal practice at the farm, and cost 0.14 and 0.18 KES m⁻² per week, respectively. Scouting that involved mite data recording was done once a week in every greenhouse and an additional weekly check was done by the scout for any upcoming pest and disease problems. Based on the scouting report, the production manager and the scout agreed on the spray

plan, and if the *T. urticae* numbers were above threshold levels a conventional spray was decided, otherwise the number of *P. persimilis* to apply was calculated based on infestation levels in the identified hotspots, and application of pesticides or predators was done within the same week as the scouting.

Insecticides were applied as top spray, minimizing side-effects on predatory mites which were protected in the lower canopy. The equipment for spraying was a central pesticide application system where pesticide preparation is done in a mixing area and then distributed via a system of pipes, and pesticides were applied using a handheld gun which breaks the spray into small droplets, and directed at the foliage. The acaricide was applied uniformly within the greenhouse and to reduce negative effects of pesticides on *P. persimilis*, they never were applied earlier than 3 days after an acaricide/insecticide treatment. The *T. urticae* infestations in the greenhouses were natural, and in all the greenhouses, another predatory mite *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) occurred naturally. Other pests requiring control included thrips (*Frankliniella occidentalis* (Pergande)), which were present in both hot-spot treated and conventional greenhouses, and aphids (*Macrosiphum rosae* (L.)), which were most

abundant in hot-spot greenhouses. Acaricides used in the trial were chosen based on a resistance management strategy set by Insecticide Resistance Action Committee (IRAC 2014) and are listed in Table 1.

Hot-spot plants, defined as plants on which scouting had identified a total of more than 40 motile mites on three leaflets, one from the base, the middle, and the top of the plant, were marked with a thin, yellow polythene strip to guide the *P. persimilis* application. Hot-spots were treated within 24 h of scouting and to ensure efficiency of mite management with *P. persimilis* the treatment included all plants within an area of 2×1 m of a flower bed, with the hot-spot tagged plant in the centre, equivalent to eight plants. The number of *P. persimilis* to apply was chosen so the *P. persimilis*:*T. urticae* ratio was 1:10, and application was focused on the affected plant(s), such that surrounding, less infested plants in the 1×2 m area received fewer mites. Hot-spots sometimes persisted for more than a week, and if the *P. persimilis* density was less than half of the *T. urticae* density after one week, additional *P. persimilis* were added.

The hot-spot treatment was introduced gradually, and the study covered the period after which all seven greenhouses assigned to this treatment had been converted. Sampling was done weekly, and each greenhouse was divided into 20 scouting stations, each station consisting of four flower beds, each 1 m wide and 100 m long, so the total area of a scouting station was 400 m². Ten plants were randomly selected from these scouting stations and marked, yielding 200 plants to be scouted within each greenhouse. All motile stages of *T. urticae* and *P. persimilis* were counted in situ with a lens of $\times 10$ magnification on three leaflets per plant, one from the canopy base (shoots bent over beds), one from the middle of the canopy (area between base and top) and one from the top (flowering shoots), amounting to a total of 600 leaflets per greenhouse. The mean number of leaflets on a plant was estimated based on a count of all leaflets in the top, middle and lower canopy of four plants and for every plant scouted, one leaflet was picked from the base canopy, middle canopy and top canopy. *T. urticae*, *P. persimilis* motiles and *N. californicus* motiles (combined adults and juveniles) were then counted using a magnifying lens, and recorded on the scouting form. Scouting was always done in the same order, and results were recorded in the same order in the scouting form, so that comparisons on pest build up

and establishment of the predatory mites could be done in each specific station. No other predatory mite species were observed, and apart from the *T. urticae* and *P. persimilis* counts data on the weekly cost of each management option and the weekly yield in terms of number of harvested rose stems was recorded and verified by the greenhouse managers and the scouts based on purchase of pesticides, *P. persimilis* abundance, and harvested stems.

Data analysis

Means of pest and predator abundances per leaflet in hot-spot treated greenhouses and in conventional greenhouses for the 30-week period of study were compared using ANOVA (Proc MIXED) (SAS Institute Inc 2008) and when necessary data were transformed prior to analysis to meet the requirements for a parametric analysis. A repeated measures analysis on weekly counts was conducted to identify any trends separating the two treatments, using a general mixed linear model (Proc MIXED) (SAS Institute Inc 2008), and because the same greenhouses were measured across time a repeated measures covariance structure was used. Treatment means were separated with a *t* test at a 95 % level of significance, and fixed effects were treatment (hot-spot or conventional), variety (seven varieties) and week (30 weeks). The full model included all interactions of fixed effects, and greenhouses together with varieties were included as a random factor. The full model was reduced backwards by removing higher-order non-significant interactions, and only retaining significant interaction effects. The same modeling approach was used for weekly costs of acaricides, cost of *P. persimilis*, and yield in terms of harvested rose stems, which were also analyzed using ANOVA (Proc MIXED). For these datasets, a repeated measures analysis was also conducted to identify any trends separating the two treatments (Proc MIXED) (SAS Institute Inc 2008), and for these datasets full models including all interaction effects were also reduced backwards until only significant interaction effects were retained.

Results

The average number of *T. urticae* per leaflet in hot-spot treated greenhouses was 3.4 times lower

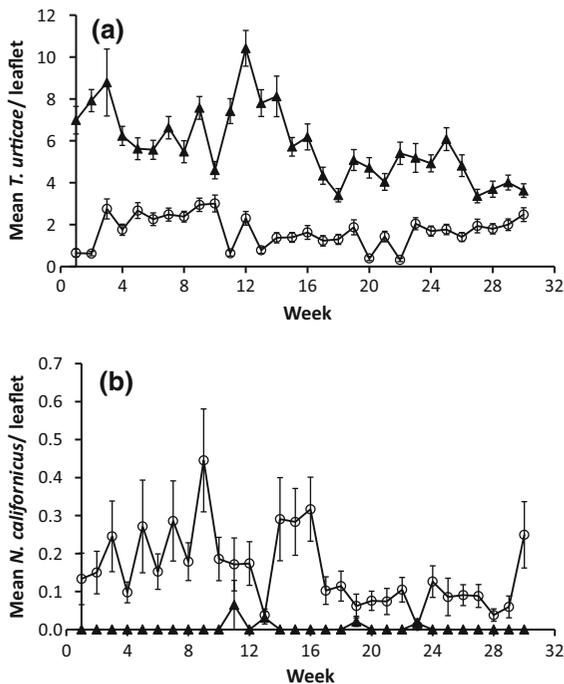


Fig. 1 Weekly mean number (\pm SE) of the motile stages of **a** *T. urticae* and **b** *N. californicus* per leaflet in hot-spot treated greenhouses (unfilled circle) and in conventional treated greenhouses (filled triangle)

(mean \pm SE) (0.57 ± 0.02) than in conventionally treated greenhouses (control) (1.93 ± 0.04) (Fig. 1a) and there was a highly significant interaction effect of treatment \times week ($F_{29, 406} = 2.3$, $P < 0.0001$) on *T. urticae* numbers (log-transformed). *P. persimilis* was only found in hot-spot treated greenhouses (0.08 ± 0.005 per leaflet), while the naturally occurring predatory mite *N. californicus* was significantly more abundant in hot-spot treated greenhouses (0.05 ± 0.004 per leaflet) than in conventional greenhouses (0.001 ± 0.0004 per leaflet) (Fig. 1b) ($F_{1, 7} = 8.8$, $P = 0.02$) (Proc MIXED, SAS Institute Inc 2008). Hot-spot treated greenhouses had on average 6.0 ± 0.29 hot-spots per week of which 2.1 ± 0.3 hot-spots per week were treated, by applying a mean of 9438.1 ± 662.3 *P. persimilis* per hot-spot. The remaining hot-spots had an adequate proportion of *P. persimilis* and were not treated. The mean number of *P. persimilis* used was 32.3 % lower in the last 15 weeks of the study compared to the first 15 weeks ($F_{1, 6} = 9.1$, $P = 0.023$).

Treatment frequency of acaricides was three times lower in hot-spot greenhouses (0.21 ± 0.03 treatments

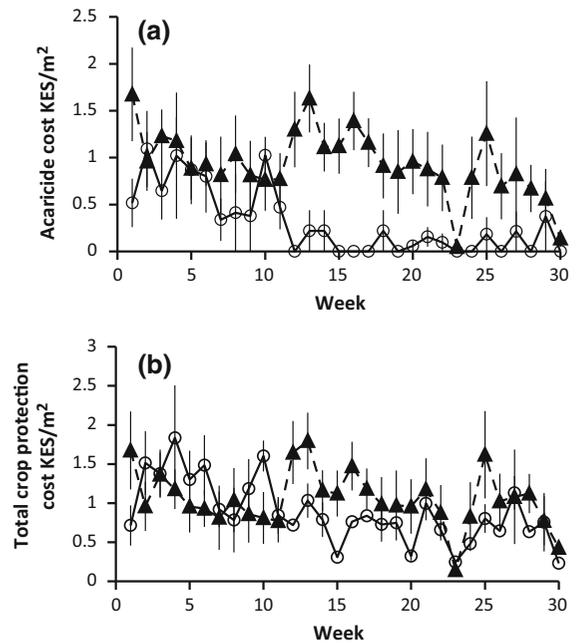


Fig. 2 Weekly mean costs (KSH) per m^2 (\pm SE) of **a** acaricide treatments and **b** the total cost of crop protection (acaricide, insecticide and *P. persimilis*) in hot-spot treated greenhouses (unfilled circle) and in conventional treated greenhouses (filled triangle)

per greenhouse per week) than in conventional greenhouses (0.64 ± 0.03 treatments per greenhouse per week) ($F_{1,6} = 35.8$, $P = 0.001$). There was no difference in use of fungicides, while insecticide use was 28 % higher in hot-spot greenhouses (0.39 ± 0.03 treatments per greenhouse per week) than in conventional greenhouses (0.28 ± 0.03 treatments per greenhouse per week) ($F_{1,6} = 9.6$, $P = 0.021$).

Cost of acaricides and other plant protection products

Amongst all pesticides, acaricide applications resulted in the highest costs, other costs were fungicides and insecticides (Fig. 2). Fungicide costs were not significantly different between treatments (data not included).

Acaricide cost was three times lower in hot-spot treated greenhouses (0.31 ± 0.05 KES m^{-2}) (1 \$US = 108 KES, Kenyan Shilling) compared with the conventionally treated greenhouses (0.94 ± 0.07 KES m^{-2}). In addition, there was a significant interaction effect of treatment and week (PROC MIXED)

($F_{29,406} = 1.8$, $P < 0.006$), reflecting that in hot-spot treated greenhouses a decrease in treatments with time was observed, with only 20 % of 45 acaricide treatments occurring in the last half of the 30 week study period. In conventional greenhouses, such a decrease was not evident, as 41 % of the 134 acaricide treatments occurred in the last half of the study. The production cost of *P. persimilis* was 15.82 ± 0.06 KES per 1000 *P. persimilis*, and in the hot-spot treated greenhouses, the average weekly cost was 0.38 ± 0.04 KES m^{-2} . The cost of controlling mites (acaricide + *P. persimilis*) was only significantly correlated with week ($F_{29,406} = 2.71$, $P < 0.0001$), with no difference in mite control costs between the two treatments. Scouting costs include the application of *P. persimilis* and any other biological control agents, and were the same in the two treatments, but personnel for spraying pesticides are hired for the whole farm so savings from reduced time spent spraying in hot-spot greenhouses could not be analyzed.

Yield effects

Weekly yield in the 14 greenhouses was assessed as harvested stems m^{-2} , and there were 4 plants m^{-2} in all greenhouses. In conventionally treated greenhouses, the mean weekly yield was 3.72 ± 0.10 stems m^{-2} and in hot-spot treated greenhouses 3.93 ± 0.10 stems m^{-2} . There was a near significant main effect of treatment on yield in favour of hot-spot treatment ($F_{1,413} = 3.8$, $P = 0.053$) and a significant effect of week ($F_{29,413} = 3.8$, $P < 0.0001$) (Proc MIXED), reflecting that harvest varied among weeks.

Pest control cost per harvested stem

The cost of spider mite control (acaricides and *P. persimilis*) for producing one harvested stem was 27 % less in the hot-spot treated greenhouses (0.69 ± 0.06 KES) than in the conventional greenhouses (0.94 ± 0.07 KES) with a highly significant main effect of treatment ($F_{1,413} = 10.8$, $P = 0.001$) and a significant effect of week ($F_{29,413} = 1.6$, $P = 0.021$). The total pest control cost to produce one harvested stem (acaricides, insecticides and *P. persimilis*) was 17 % less in hot-spot treated greenhouses (0.88 ± 0.07 KES) than in conventional greenhouses (1.06 ± 0.07 KES), with significant main

effects of treatment ($F_{1,7} = 6.6$, $P = 0.037$) and week ($F_{29,406} = 1.6$, $P = 0.034$).

Discussion

We show that hot-spot treatments with *P. persimilis* are an effective method to reduce infestations of *T. urticae* on roses in commercial greenhouses, resulting in a low requirement for application of synthetic pesticides on an annual basis, and such acaricide application can be based on a monitoring schedule and mite action threshold levels. In contrast, continuous, though controlled, use of synthetic acaricides could not maintain *T. urticae* infestation at levels comparable with hot-spot application of *P. persimilis*. The decrease in acaricide treatments over time after the onset of hot-spot treatment is a general experience at the farm, and situations where few acaricide treatments are needed are normally achieved. There was also a decrease in the number of *P. persimilis* applied over time in the hot-spot greenhouses, and this indicates that our results to some extent represent a conversion period from acaricides to biological control for the hot-spot greenhouses, and that spider mite control using lower *P. persimilis* input can be achieved. More aphids were observed in hot-spot greenhouses, and may have caused the higher insecticide use. This is an undesirable effect and biological control agents are available which could replace insecticides against aphids. However, total use of acaricides and insecticides remained significantly lower in the hot-spot greenhouses, with a total of 9.1 treatments over the study period, compared to 13.8 treatments in the conventional greenhouses (Table 2).

Acaricide use for management of *T. urticae* is not only common in Kenyan commercial greenhouses, but also in other parts of the world (Bugeme et al. 2008; Knapp et al. 2006). However, *T. urticae* is known to develop resistance to most widely used acaricides (Goka 1998; Stavrinides et al. 2009; Khajehali et al. 2011; van Leeuwen 2011), leading to ineffective control.

We did not test for acaricide resistance in this study, but the lack of mite reduction in conventional greenhouses to levels below threshold limits may be an indication of resistance. Hence, year-round utilization of acaricides as a sole response to *T. urticae* may not offer long term control of the pest in protected cultivation.

Our findings show that hot-spot management of *T. urticae* can significantly reduce the costs of rose production, thus providing an economic incentive for growers to change to this method of control, and this result is supported by an unreplicated experiment in an Austrian commercial rose production (Blümel et al. 2002). The observed difference in *N. californicus* density between hot-spot greenhouses and conventional greenhouses could be a result of the hot-spot greenhouses being gradually introduced over a period of 1–5 months before the onset of the study period, allowing *N. californicus* to recover in numbers. The density of *N. californicus* in hot-spot treated greenhouses was so high (0.05 mites per leaflet) that it can have significantly contributed to spider mite control, making it in theory impossible to ascribe biocontrol to *P. persimilis* (0.08 mites per leaflet) alone, but perhaps rather to a combined effect of reduced acaricides input, conserving naturally occurring *N. californicus*, and the effect of hot-spot application of *P. persimilis*. In a study investigating traditional rose production system with a closed canopy, allowing easy dispersal of mites, long-term stability of spider mites and predatory mites was found (Gough 1991). However, modern systems are more intensive, the canopy is more open, and such stability in mite populations is not found. Pesticide use can lead to failure of biological control, and an attempt to demonstrate the effectiveness of *P. persimilis* and *N. californicus* use in commercial greenhouses failed due to the use of pesticides (De Vis and Barrera 1999). In agreement with our findings, an IPM study in roses assessing application of *P. persimilis* based on co-occurrence with spider mites resulted in a good spider mite control (Casey et al. 2007), but lacks pesticide use information. The present study from Kenya demonstrates effective hot-spot management of *T. urticae* in large commercial greenhouses, though having a production plant on the farm that supplies predatory mites at production cost is an exception. Assuming harvested stems were of at least similar quality (data could not be provided due to commercial interests), this demonstrates that it is possible to considerably decrease acaricide use with hot-spot treatments with *P. persimilis*, which is positive as hot-spot treatment provides important benefits economically as well as environmentally and for workers health. Finally, less use of acaricides and a move towards reduced risk pesticides will better protect beneficial species such as

P. persimilis and *N. californicus* (Nicetic et al. 2001; Numa et al. 2011). However, the system depends on intensive scouting. In countries with higher labour costs, e.g. in Europe, the situation may be different, and such intensive scouting not possible. Future research could further improve the method, and for example studies to establish the needed proportion of *P. persimilis* to *T. urticae* in a hot-spot may help make the use of predators more economically feasible (Park et al. 2000; Hilarion et al. 2008; Alatawi et al. 2011). Likewise, a higher efficiency of *P. persimilis* may be obtained if combined with *N. californicus* (Blümel et al. 2002), and when a more full biological control strategy including biological control of other pests is developed (Casey et al. 2007).

However, hot-spot treatments can only be adopted by farmers if effective relative to conventional (prophylactic, blanket application) applications, hot-spot treatments rely heavily on scouting and monitoring to help detection of pests, and should be based on pest threshold levels, considering threat to the crop, and cost-benefit analysis of the control option (Zehnder et al. 2007). In conclusion, hot-spot treatments allow for large-scale commercial rose production with the same productivity as in conventionally treated roses, while reducing the cost of pest control, particularly by cutting acaricide costs.

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