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Native phytoseiid mites as indicators of non-target effects of the introduction of *Typhlodromalus aripo* for the biological control of cassava green mite in Africa

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Abstract

The need to evaluate non-target effects of classical biological control of arthropod pests has received considerable attention in recent years. We determined with repeated field surveys the changes in abundance and distribution of the phytoseiid mite fauna in cassava fields resulting from the introduction of the neotropical phytoseiid *Typhlodromalus aripo* into two countries—Malawi and Mozambique—in southern Africa for the biological control of *Mononychellus tanajoa*. *Typhlodromalus aripo* abundance was similar, while the abundance of the target pest *M. tanajoa* declined progressively, during the 2 years after the introduction of *T. aripo* into the target countries. We did not detect any changes in the abundance of the most common native phytoseiids mites—*Euseius baetae*, *Euseius bwende* and *Ueckermannseius saltus*—on cassava in Mozambique. In contrast, the abundance of two of the most common native phytoseiids, *Euseius fustis* and *Iphiseius degenerans*, on cassava in Malawi were apparently facilitated—i.e., their abundance was enhanced by the introduction of *T. aripo;* while the abundance of a third species, *U. saltus*, was not affected. For only one species, *E. baetae*, within-plant distribution shifted to the lower parts of the cassava canopy as a result of the introduction of *T. aripo*, which resides in the upper parts of the cassava canopy, but without any measurable negative effects on biological control of *M. tanajoa.* While the overall abundance of phytoseiid mites found on non-cassava vegetation was not affected by *T. aripo* introduction, there were some changes in relative abundance of some species in Malawi. Possible mechanism for the increase in abundance of *I. degenerans* and *E. fustis* in Malawi, and changes in within-plant distribution of *E. baetae* and *I. degenerans* in Mozambique and Malawi, respectively, are discussed. The methodology developed for assessing potential non-target effects of *T. aripo* introduction into southern Africa has significantly advanced classical biological control efforts against an economically important cassava pest.

Keywords: Acari; Tetranychidae; Phytoseiidae; *Mononychellus tanajoa*; Classical biological control

1. Introduction

Classical biological control is a strategy of biological control that consists of the introduction of suitable exotic natural enemies for permanent establishment in the new environment and for self-sustained suppression of target

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pest populations below economically damaging levels (Eilenberg et al., 2001). Classical biological control has been implemented for over a century and has resulted in enormous economic returns while providing by and large an environmentally safe alternative to chemical control of pests and diseases (see reviews by Hall et al., 1980; Wratten and Gurr, 2000; van Lenteren et al., 2003; Alene et al., 2005). Despite the successes there are some high profile cases of disputably undesirable negative effects, which have encouraged biological control practitioners to give special attention to non-target effects of exotic natural enemy introductions (e.g., Howarth, 1991; Simberloff and Stiling, 1996; Cory and Myers, 2000; Follett et al., 2000; Lynch and Thomas, 2000; Waage, 2001; Simberloff, 2004; see also van Lenteren et al., 2003, 2006 for a comprehensive framework for the assessment of environmental risk of classical biological control). Negative non-target effects often arise because of neglect in proper pre-introduction testing and/or lack of sufficient knowledge of the breadth of the food web with which the alien species might interact, particularly prior to alien enemy introductions (Louda et al., 2003). Central to these issues is the choice of appropriate and representative non-target species, which is often compounded by difficulties in measuring indirect effects on the non-target species, as indirect effects often depend on the position of a species within the trophic hierarchy in a particular system (van Lenteren et al., 2003, 2006). Knowledge of the trophic position of a particular species requires sufficient baseline information of the food web, which is often obtained, if at all, only after natural enemy introductions.

Non-target effects of classical biological control arise when alien natural enemies attack native or other non-target species of plants and animals (herbivores and/or predators) and disrupt ecological balance (Ehler, 1999; van Lenteren et al., 2003). Studies on non-target effects are very rare in classical biological control (van Lenteren et al., 2003) and most of these studies were conducted after the introductions, and establishment of the natural enemies. To our knowledge, there are no case studies on non-target effects of the introduction of exotic phytoseiid mites, a group of predators widely used in biological control of mite pests of agricultural crops. The classical biological control campaign of the cassava green mite *Mononychellus tanajoa* (Bondar) in southern Africa provided an opportunity to examine the non-target effects of the introduction of the neotropical phytoseiid mite *Typhlodromalus aripo* DeLeon on the native phytoseiid fauna found in cassava habitat in southern Africa.

Typhlodromalus aripo was first introduced from Brazil into the Republic of Benin (West Africa) in 1993 for the biological control of *M. tanajoa*, which is also of neotropical origin (Hanna and Toko, 2003; Yaninek and Hanna, 2003; Hanna et al., 2005). While pre-introductory surveys were conducted in several countries in sub-Saharan Africa to determine the composition of native phytoseiid species occurring on cassava plants and their associated vegetation (e.g., Yaninek and Onzo, 1988), the available information could not be used to properly evaluate non-target effects of *T. aripo* introduction in West and Central Africa, as data on the abundance and spatial distribution of the native phytoseiids were not available. An opportunity to properly conduct non-target effect studies arose with the planned introductions of *T. aripo* into southern Africa.

Typhlodromalus aripo was first introduced into Mozambique in 1999 and into Malawi in 1998 (Hanna and Toko, 2003) for the biological control of *M. tanajoa*. This predator resides in the apex (growing point) of cassava shoots (Bakker, 1993) during the day light hours, and migrates at night down to the leaves where the *M. tanajoa* and native phytoseiid mites are found (Onzo et al., 2003b). Surveys conducted before the introduction of *T. aripo* revealed that *Euseius baetae* (Meyer and Rodrigues), *Euseius bwende* (Pritchard and Baker), and *Ueckermannseius* (= *Typhlodromalus*) *saltus* (Denmark and Muma) are the most common native phytoseiid species on cassava in Mozambique; while *Euseius fustis* (Pritchard and Baker), *Iphiseius degenerans* (Berlese) and *U. saltus* are the most common phytoseiid species encountered on cassava in Malawi (Zannou et al., 2005b). It is not known if *E. baetae* and *E. bwende* feed on *M. tanajoa*, but the other four species are all known to feed, reproduce and survive on *M. tanajoa* (Akpokodje et al., 1990; Bruce-Oliver et al., 1996; Nwilene and Nachman, 1996; Gnanvossou et al., 2003b; B. Ojo and J.S. Yaninek, unpublished data). That *T. aripo* and native phytoseiids share food and space on the cassava plant provides the basis for the assumption that they might compete for resources or interact directly—e.g. through intraguild predation—with species- or community-level consequences for their respective abundance and distribution, as well as their impact on the target pest, *M. tanajoa*.

As a first step in our efforts to determine the non-target effects of *T. aripo* introduction into southern Africa, we conducted studies on individual level interactions among the alien and native phytoseiids in a laboratory setting (Zannou et al., 2005a). These intraguild predation experiments showed that *T. aripo*, *E. fustis* and *I. degenerans* feed on heterospecific larvae and protonymphs in the presence or absence of food (*M. tanajoa* and pollen). This evidence of interspecific predation among the native and the alien phytoseiids suggested that these interactions might affect, directly or indirectly, the abundance of native phytoseiids in suppressing *M. tanajoa* populations. Together, available knowledge of niche and diet breadth of *T. aripo* and native phytoseiids, their distributions, and their individual-level interactions provided considerable baseline information on the characteristics of the cassava-mite food web in cassava fields. It is against this extensive background knowledge that we base our choice of native phytoseiid mites as a target group for determining non-target effects of classical biological control of cassava green mite in southern Africa.

The main objective of this paper is to determine the non-target effects of *T. aripo* introduction into southern Africa, by measuring changes in the abundance and distribution of the most common native phytoseiids occurring on cassava and its associated vegetation. To determine this impact, we compared, on cassava and associated vegetation, the abundance and distribution of phytoseiid fauna before the introduction of *T. aripo* with their abundance and distribution at one and two years after the introduction of *T. aripo*.

2. Materials and methods

2.1. Sites selection and survey periods

Surveys were conducted in Mozambique and Malawi before, and 1 and 2 years after *T. aripo* introduction. Survey sites were selected across the main cassava growing areas in both countries. For logistical reasons, sites were selected along main roads in Mozambique and along main and secondary roads in Malawi. Four survey areas were selected in Mozambique: (1) Vilankulo (21–22°S and 35–36° E, costal savanna with less than 800 mm of annual rainfall, Inhambane province), (2) Mitilili (15°25′–15°40′S, 38°10′–38°20′E, continental transition forest with more than 1200 mm of annual rainfall, Zambezia province), (3) Muecate (14°50′– 15°10′S, 39°–40°E, continental forest with more than 1200 mm of annual rainfall, Nampula province), and (4) Nacala-Velha (14–16°S and 40–41°E, coastal savanna with less than 1000 mm of annual rainfall, Nampula province).

Three survey areas were selected in Malawi: (1) Salima-Nkhotakota (Salima at 13°40′–13°45′S and 34°34′–34°36′E, and Nkhotakota at 12°52′–12°58′S and 34°15′–34°18′E; both are coastal savanna with less than 1300 mm of annual rainfall, Central province), (2) Nkhata Bay-Mzuzu (11°28′–11°51′S and 34°00′–34°12′E, rainforest with more than 1700 mm of annual rainfall, Central province) and (3) Chiweta-Mlowe (10°42′–10°47′S and 34°10′ and 34°13′E, coastal savanna with less than 1200 mm of rainfall, Northern province). In each country, three to 10 fields were sampled in each site during each visit. Pre-introduction surveys were conducted in Mozambique in April 2000, 2001 and 2002 (end of rainy season) and in Malawi in May 2000 (end of rainy season). Geographical position of each site and each field was determined with a handheld GPS (Magellan 2000 XL).

In Malawi, post-introduction surveys were conducted in May 2001 and 2002 in the fields visited during the pre-introduction survey and colonized by *T. aripo*. Because of problems with the persistence of *T. aripo* in the Salima site in Malawi, fields at this site were not included in the post-introduction surveys, and were replaced by fields from the Nkhotakota site. The same fields were visited at each site during each of the surveys; except those that were harvested by the time we arrived for the following survey. In such cases, a nearby field was selected to replace the harvested field. Surveyed fields in each country were grouped by time of *T. aripo* presence—i.e., before introduction, and first and second year of *T. aripo* presence. Other alternative approaches to field selection were not possible due to reasons stated below.

In Mozambique, post-introduction surveys were conducted in April 2001 and 2002. Sixteen fields located in Vilankulo, Mitilili, Muecate, and Nacala-Velha were surveyed before the introduction of *T. aripo*; while 15 and 7 fields were visited after 1 and 2 years of *T. aripo* presence, respectively. In Malawi, 15 fields located in Salima-Nkhotakota, Nkhata-Bay-Mzuzu, and Chiweta-Mlowe were visited before the introduction of *T. aripo*, while 17 fields, located in Nkhotakota, Nkhata-Bay-Mzuzu, and Chiweta-Mlowe were surveyed for the first year of *T. aripo* presence, and 12 fields located in Nkhotakota, Nkhata-Bay-Mzuzu, and Chiweta-Mlowe were visited for the second year of *T. aripo* presence. Difficulties in finding fields adjacent to previously surveyed fields led to differences in the total number of fields during the second and third year in Mozambique and during the third year in Malawi.

The experimental design followed in this study relies on comparisons of phytoseiid abundance and distribution before *T. aripo* introduction—representing fields without *T. aripo*—and 1 and 2 years after *T. aripo* introduction—representing fields with *T. aripo*. A more preferred approach would have been the use of paired comparisons, during each year of the study, of cassava fields with and without *T. aripo* and located behind, inside and ahead of the moving front of the predator to minimize between field variability due to location within the surveyed area. This option was not possible, however, due to the speed with which *T. aripo* colonized new areas. Under these *T. aripo* dispersal conditions, fields with or without *T. aripo* would have been at least 100 km apart, which is the speed at which *T. aripo* spreads within a year (Yaninek and Hanna, 2003; Hanna et al., manuscript in preparation). Those ‘control’ no-*T. aripo* fields would have been likely very different from the fields with 1 or 2 years of *T. aripo*, which for obvious reasons would not have been appropriate for the comparisons intended by our study. A second approach would have been to physically or chemically exclude *T. aripo* from fields adjacent to fields with *T. aripo* over a 2-year period. That would have necessitated pesticide applications (as physical exclusion is impossible to achieve constantly and consistently) over at least a 24-months period—the average cassava crop cycle is 12 months—and covering large distances in the two countries, which would have made such effort unpractical. These aforementioned difficulties necessitated the approach we followed in this study—i.e., survey of fields in the same areas 1 year before *T. aripo* introduction and at 1 and 2 years after *T. aripo* had colonized the fields. We minimized between-field differences by selecting the same or adjacent fields during each year of the surveys.

2.2. Mite abundance and distribution

In each survey field, 30 cassava plants were selected randomly for the mite census. From each plant, one apex (growing point), and one leaf from each of the top (i.e., the fourth to sixth leaf from the apex), the middle (i.e. the 12th to 15th leaf from the apex) and the bottom (i.e. one of the last three leaves from the apex) stratum of the plants were sampled. Apices were placed individually in vials containing 70% ethanol and brought to the laboratory where all mites present were counted, mounted on slides, and identified to species. Cassava leaves were inspected directly in the field with the aid of a binocular microscope. All mites found on the abaxial surface of leaves were counted and recorded by species. Except for *M. tanajoa*, all other mobile stages of other mite species were collected and preserved in 70% ethanol for further identification in the laboratory.

In addition to inspecting cassava plants, the five visually most common plant species found in the surveyed cassava fields were inspected. At least three plants of each species separated by a minimum of 5 m were selected. The abaxial leaf surfaces of each of the selected plant species was inspected under a binocular microscope for 15 min. In addition, 30 apices (if present) of each sampled plant species were collected, placed in 70% ethanol and brought to the laboratory for separation, counting, and identification of mites. Plant species were identified directly in the field, using reference books (Palgrave, 1983; Vernon, 1983; Akobundu and Agyakwa, 1987) or when needed, with the assistance of botanists in each respective country.

2.3. Statistical analyses

For each country, mean densities of *T. aripo* per apex were transformed using Log_{10}(mean density + 1). *T. aripo* densities at 1 and 2 years of its presence were compared using PROC TTEST (SAS, 1999).

To determine the effects of *T. aripo* introduction on the common native phytoseiid species on cassava plants in each country, analysis of variance (PROC GLM; SAS, 1999), was applied to log-transformed mean densities (top, middle and bottom leaves together) of each of the three most common phytoseiid species recorded before, and 1 and 2 years after *T. aripo* introduction, with densities of *T. aripo* as a covariate. For each country and for a given phytoseiid species, mean densities were compared among survey periods with least square means (LSMEANS). Analysis of variance was also applied to the densities of native phytoseiid species to compare densities of the most common phytoseiid species in each country during each survey period. For each country, phytoseiid densities were compared within each survey period and between survey periods with LSMEANS.

Similarly, to determine the impact of *T. aripo* introduction on native phytoseiids inhabiting the five visually most common plant species (other than cassava) found in cassava fields, we used mean densities per 75 min (total sampling time per field) and per survey period for each of the four most common phytoseiids recorded before, and one and two years after the introduction of *T. aripo*. Densities of each species were log-transformed and used in analysis of variance PROC GLM and LSMEANS (SAS, 1999) to compare phytoseiid densities within each survey period and between survey periods.

Finally, the impact of the introduction of *T. aripo* on the distribution of native phytoseiids within cassava plants was determined by comparing in each country the distribution of each species within-cassava plant strata before the introduction, and after 1 and 2 years of *T. aripo* presence. For each survey period, PROC MIXED-REML (SAS, 1999) was applied to log-transformed mean densities per leaf stratum (top, middle or bottom leaf) of each of the most common phytoseiid species. Strata and fields represented the random and fixed factors, respectively. Mean densities of each common phytoseiid species were compared within cassava strata using LSMEANS. We did not include cassava terminal shoot because native phytoseiids are rarely found on that plant part.

3. Results

3.1. Abundance of T. aripo

In Mozambique, *T. aripo* densities were similar during the first and second year of its presence ($t = 1.32$, $P = 0.202$, $n = 22$), and ranged between 1.70 and 5.30 actives per apex during the first year, and between 0.90 and 3.53 actives per apex during the second year of its presence. As in Mozambique, mean *T. aripo* densities in Malawi were similar during the first and second year of its presence ($t = 0.12$, $P = 0.905$, $n = 29$) and ranged from 0.07 to 3.33 during the first year and 0.10 to 10.77 during the second year of its presence.

Except in Muecate (Mozambique), where six individuals of *T. aripo* were collected from terminal shoots of *Cajanus cajan* (L.) Millsp. (Fabaceae) and flowers of *Tridax procumbens* L. (Asteraceae) during the 2001 survey (1 year after *T. aripo* presence), *T. aripo* was not found on any plants associated with cassava in Mozambique and Malawi.

3.2. Abundance of native phytoseiids on cassava

In Mozambique, comparisons of mean densities of each phytoseiid species within a given survey period showed that densities of *E. baetae* were significantly higher than those of *E. bwende* and *U. saltus* for all the three survey years (Table 1a). However, mean densities of each of these phytoseiid species were similar in all survey periods, except for those of *U. saltus* in the first and second years of presence of *T. aripo*, which were higher than in the absence of *T. aripo* (Table 1a). Analysis of variance applied to mean densities per leaf of each of *E. baetae*, *E. bwende* and *U. saltus*, with densities of *T. aripo* as covariate, showed that the presence of *T. aripo* (principal variable) and densities of *T. aripo* (covariate) had no significant impact on the densities of *E. baetae*, *E. bwende* and *U. saltus* (Table 2).

In Malawi, mean densities of *E. fustis*, *I. degenerans* and *U. saltus*, the three most common native phytoseiid mites on cassava, were similar within each of the three survey periods (Table 1b). Mean densities of each of these phytoseiid species were likely similar in all survey periods,

Table 1
Comparisons of densities[a] (means ± SE) of the most common native phytoseiids inhabiting cassava during surveys conducted in Mozambique and Malawi before and after the introduction of *Typhlodromalus aripo*

T. aripo presence	Phytoseiid mite species[b]			*F*-test [*F*, *P* (df)]
	E. baetae	*E. bwende*	*U. saltus*	
(a) Mozambique				
Absent	0.52 ± 0.15c	0.07 ± 0.03b	0.02 ± 0.01a	12.8, <0.001 (2,45)
Present 1 year	0.51 ± 0.14c	0.07 ± 0.04b	0.05 ± 0.02a*	11.1, <0.001 (2,42)
Present 2 years	0.54 ± 0.24b	0.07 ± 0.05a	0.10 ± 0.04a*	3.70, 0.045 (2,18)
F, *P* (df)	0.00, 0.998 (2,35)	0.01, 0.987 (2,35)	3.95, 0.028 (2,35)	
	Phytoseiid mite species[b]			
	E. fustis	*I. degenerans*	*U. saltus*	
(b) Malawi				
Absent	0.54 ± 0.13	0.31 ± 0.14	0.19 ± 0.09	2.32, 0.111 (2,42)
Present 1 year	1.19 ± 0.36	0.63 ± 0.26*	0.50 ± 0.32	2.00, 0.146 (2,48)
Present 2 years	1.10 ± 0.33	1.98 ± 1.10*	0.29 ± 0.16	3.01, 0.063 (2,33)
F, *P* (df)	1.00, 0.378 (2,41)	3.30, 0.047 (2,41)	0.27, 0.767 (2,41)	

[a] Means are per leaf for pooled data of top, middle and bottom leaves.

[b] Within rows (comparisons across species), means with different letters were significantly different ($P < 0.05$; GLM; LSMEANS). Within columns (comparisons across periods of *T. aripo* presence), mean densities followed by an * of *U. saltus* in Malawi and *I. degenerans* in Mozambique at 1 and 2 years of *T. aripo* presence were significantly greater than the respective mean densities of each species when *T. aripo* was absent ($P < 0.05$; GLM; LSMEANS).

Table 2
Summary ANOVAs of the effect of the presence (or absence) of *T. aripo*—as principal independent variable and densities of *T. aripo* as co-variable—on densities of the most common native phytoseiid species inhabiting cassava in Mozambique and Malawi

Sources of variation[a]	Num df[b]	*F*	*P*
Mozambique			
Dependant variable: *Euseius baetae*			
Presence or absence of *T. aripo*	2	0.04	0.959
Density of *T. aripo*	1	0.11	0.741
Dependant variable: *Euseius bwende*			
Presence or absence of *T. aripo*	2	0.71	0.501
Density of *T. aripo*	1	2.41	0.130
Dependant variable: *Ueckermannseius saltus*			
Presence or absence of *T. aripo*	2	3.17	0.055
Density of *T. aripo*	1	2.85	0.101
Malawi			
Dependant variable: *Euseius fustis*			
Presence or absence of *T. aripo*	2	3.47	0.041
Density of *T. aripo*	1	8.50	0.006
Dependant variable: *Iphiseius degenerans*			
Presence or absence of *T. aripo*	2	3.93	0.028
Density of *T. aripo*	1	1.22	0.276
Dependant variable: *Ueckermannseius saltus*			
Presence or absence of *T. aripo*	2	0.46	0.634
Density of *T. aripo*	1	8.18	0.007

[a] Presence or absence of *T. aripo*: first year of presence, second year of presence or absence of *T. aripo*.

[b] Numerator degrees of freedom. Error degrees of freedom are 34 and 40 for Mozambique and Malawi, respectively.

except for those of *I. degenerans* in the first and second year of presence of *T. aripo*, which were higher than in the absence of *T. aripo* (Table 1b). Analysis of variance applied to mean densities per leaf of each of *E. fustis*, *I. degenerans* and *U. saltus*, with presence of *T. aripo* as principal variable and densities of *T. aripo* as covariate, showed that the presence of *T. aripo* and the densities of this species had all positive impacts on densities of *E. fustis* at 1 and 2 years of *T. aripo* presence (Table 2). Moreover, the presence of *T. aripo* had a positive impact on densities of *I. degenerans* at 2 years of *T. aripo* presence (Table 2), and densities of *T. aripo* had a positive impact on densities of *U. saltus* at 1 year of *T. aripo* presence (Table 2).

3.3. Abundance of native phytoseiids on non-cassava vegetation

Table 3 presents the statistical analyses of mean densities of the four most common phytoseiid species recorded on plants associated with cassava in Mozambique and Malawi before *T. aripo* introduction, and at 1 and 2 years of *T. aripo* presence.

In Mozambique, of the four most common phytoseiid species—*Amblyseius sundi* Pritchard and Baker, *Paraphytoseius horrifer* (Pritchard and Baker), *Typhlodromips shi* (Pritchard and Baker), and *E. baetae*—the latter was the most abundant phytoseiid species encountered on plants associated with cassava before *T. aripo* introduction (Table 3a) and 1 year after *T. aripo* presence (Table 3); however, mean density of each of the four species did not change over the three survey periods (Table 3a).

In Malawi, the four most common phytoseiid species occurring on vegetation in cassava habitat—*Euseius magucii* (Meyer and Rodrigues), *Euseius orygmus* (Ueckermann and Loots), *I. degenerans*, and *T. shi*—were present in equal abundance before *T. aripo* introduction (Table 3b). That changed, however, after *T. aripo* introduction, whereby *I. degenerans* became the most common phytoseiid species at 1 year of *T. aripo* presence (Table 3b), and *I. degenerans* and *E. orygmus* became the most abundant species at 2 years of *T. aripo* presence (Table 3b). Mean density of *E. magucii*, *E. orygmus* and *T. shi* did not change over the

Table 3
Comparisons of densities[a] (means ± SE) of the four dominant native phytoseiids on plants associated with cassava in relation to years of *Typhlodromalus aripo* presence in (a) Mozambique and (b) Malawi

T. aripo presence	Phytoseiid mite species[b]				*F*-test [*F*, *P* (df)]
	A. sundi	*E. baetae*	*P. horrifer*	*T. shi*	
(a) Mozambique					
Absent	3.9 ± 1.7a	19.6 ± 4.7b	3.0 ± 1.2a	2.0 ± 1.0a	9.88, <0.001 (3,60)
Present 1 year	2.2 ± 0.9a	10.9 ± 2.9b	4.9 ± 1.9ab	2.6 ± 1.1a	2.98, <0.039 (3,56)
Present 2 years	3.7 ± 2.0	10.4 ± 4.6	4.4 ± 2.1	2.4 ± 1.8	1.34, 0.286 (3,24)
F, *P* (df)	0.13, 0.867 (2,41)	1.19, 0.317 (2,41)	0.22, 0.805 (2,41)	0.12, 0.884 (2,41)	
	Phytoseiid mite species[b]				
	E. magucii	*E. orygmus*	*I. degenerans*	*T. shi*	
(b) Malawi					
Absent	1.4 ± 0.6	4.0 ± 1.6	14.1 ± 6.0	3.6 ± 2.0	1.92, 0.136 (3,56)
Present 1 year	1.8 ± 0.7a	10.2 ± 2.8b	29.8 ± 8.7c*	2.0 ± 1.3a	10.61, <0.01 (3,64)
Present 2 years	3.8 ± 1.5a	18.9 ± 5.2b	31.7 ± 8.5c*	3.6 ± 2.2a	9.51, <0.01 (3,44)
F, *P* (df)	1.63, 0.208 (2,35)	2.53, 0.092 (2,35)	3.78, 0.031 (2,35)	0.12, 0.884 (2,35)	

[a] Means are per 75 min of plant inspection per field.

[b] Within rows and for each country, mean densities with different letters are significantly different at $P < 0.05$; GLM; LSMEAN). Within columns and in Malawi, mean densities of *I. degenerans* followed by an * at 1 and 2 years of *T. aripo* presence were significantly greater than its mean density when *T. aripo* was absent ($P < 0.05$; GLM; LSMEAN).

three survey periods (Table 3b); however, *I. degenerans* occurred in greater abundance at 1 and 2 years of *T. aripo* presence compared with its abundance prior to *T. aripo* introduction (Table 3b).

3.4. Within-plant distribution of phytoseiid mites on cassava

In addition to changes in the abundance of native phytoseiids at the field level, we were also interested in documenting changes in the within-plant distribution of the phytoseiids as affected by *T. aripo* introduction. In Mozambique, mean densities of *E. baetae* before *T. aripo* introduction were lowest on top leaves, intermediate on middle leaves and highest on bottom leaves ($F_{2,45} = 8.83$, $P < 0.001$; Fig. 1). After *T. aripo* introduction, mean densities of *E. baetae* became similar on middle and bottom leaves but significantly higher on those strata than on the top leaves (one year of *T. aripo* presence: $F_{2,42} = 8.98$, $P < 0.001$; 2 years of *T. aripo* presence: $F_{2,18} = 3.75$, $P = 0.044$). Mean densities of *E. bwende* and *U. saltus* did not differ among plant strata, except that *U. saltus* was more abundant on middle leaves compared with top and bottom leaves during the first year after *T. aripo* presence (*E. bwende*—before *T. aripo* introduction: $F_{2,45} = 1.90$, $P = 0.159$; 1 year of *T. aripo* presence: $F_{2,42} = 2.73$, $P = 0.077$; and 2 years of *T. aripo* presence: $F_{2,18} = 1.68$, $P = 0.215$; *U. saltus*—before *T. aripo* introduction: $F_{2,45} = 2.04$, $P = 0.140$;

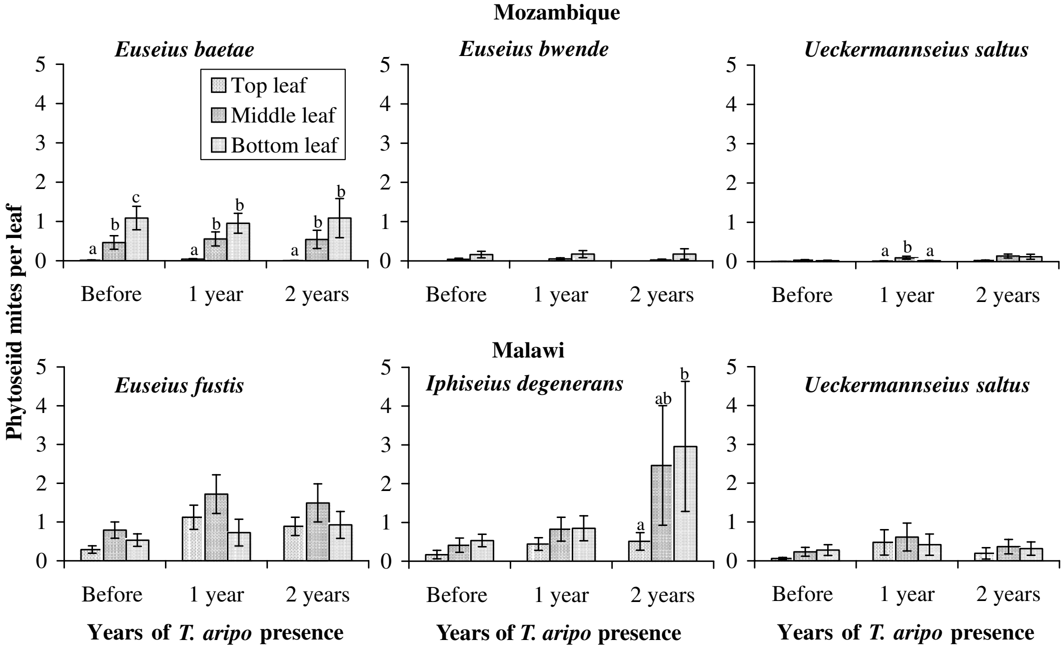

Fig. 1. Within-plant distribution of native phytoseiids on cassava in Mozambique and Malawi in relation to years of *T. aripo* presence. For a given period and a given species, mean densities with different letters are significantly different at $P < 0.05$ (MIXED-REML, LSMEAN).

1 year of *T. aripo* presence: $F_{2,42} = 3.93$, $P = 0.027$; and 2 years of *T. aripo* presence: $F_{2,18} = 1.72$, $P = 0.207$; Fig. 1).

In Malawi, *E. fustis* and *U. saltus* were equally distributed among the three plant strata during the three years of the study (*E. fustis*—before *T. aripo* introduction: $F_{2,42} = 2.70$, $P = 0.076$; 1 year of *T. aripo* presence: $F_{2,48} = 0.49$, $P = 0.615$; and 2 years of *T. aripo* presence: $F_{2,33} = 0.53$, $P = 0.593$; *U. saltus*—before *T. aripo* introduction: $F_{2,42} = 1.34$, $P = 0.270$; 1 year of *T. aripo* presence: $F_{2,48} = 0.20$, $P = 0.822$; and 2 years of *T. aripo* presence: $F_{2,33} = 0.31$, $P = 0.739$; Fig. 1). *I. degenerans*, however, occurred in larger numbers on bottom leaves compared with top and middle leaves in the second year of presence of *T. aripo* (*I. degenerans*—before *T. aripo* introduction: $F_{2,42} = 0.98$, $P = 0.382$; 1 year of *T. aripo* presence: $F_{2,48} = 0.67$, $P = 0.514$; and 2 years of *T. aripo* presence: $F_{2,33} = 3.70$, $P = 0.043$).

4. Discussion

To our knowledge this study represents the first detailed investigation of non-target effects of classical biological control using phytoseiid predators. Our results clearly show that the introduction of *T. aripo* did not negatively affect densities or species composition of native phytoseiids commonly found on cassava in Mozambique and Malawi for a 2-year period after *T. aripo* colonization of cassava fields in the surveyed areas of the two countries. There were, however, some indications that *T. aripo* presence facilitated two native phytoseiid species as shown by greater abundance of *I. degenerans* and *E. fustis* in Malawi on cassava plants after *T. aripo* introduction compared with their abundance prior to *T. aripo* introduction.

The results from the 3 years of field surveys contrast sharply with expectations from laboratory results, which showed common and high interspecific predation by *T. aripo*, *E. fustis* and *I. degenerans* (Zannou et al., 2005a), all type IV generalist predators (McMurtry and Croft, 1997). The laboratory data (Zannou et al., 2005a) and the 3 years of field data presented in this article clearly indicate that individual-level tests under laboratory conditions were not appropriate predictors of non-target effects of alien enemy introductions. Two reasons may explain the absence of negative effects of the introduction of *T. aripo* on abundance and within-plant distribution of the most common native phytoseiids inhabiting cassava plants. First, *T. aripo* and native phytoseiids inhabit different parts of the cassava plant, although their diets overlap considerably (Akpokodje et al., 1990; Bruce-Oliver et al., 1996; Nwilene and Nachman, 1996; McMurtry and Croft, 1997; van Rijn and Tanigoshi, 1999; Gnanvossou et al., 2003b, 2005). *T. aripo* resides mainly in terminal shoots of cassava plants (Bakker, 1993) during the daylight hours (Onzo et al., 2003b) and forages on the top 10–20% of the cassava plant canopy during the night hours (Onzo et al., 2003b). Moreover, *T. aripo* is restricted to cassava while at least three of the native phytoseiids occur both on cassava and associated vegetations (Zannou et al., 2005b). In contrast, native phytoseiids on cassava inhabit mainly middle and bottom leaves of the cassava plant (Zannou et al., present paper; B. Ojo and J.S. Yaninek, IITA, unpublished data). This partial temporal and habitat segregation may minimize encounters between *T. aripo* and native phytoseiids, and reduce negative interactions (i.e., competition, interferences, etc.) that could result from sharing the same prey (e.g., *M. tanajoa* and other foods such as various plant pollens and exudates). Second, the phytoseiid species found on cassava could avoid encounters with other predators by moving to cassava leaves or plants that are not yet occupied by their competitors (Gnanvossou et al., 2003a). These authors reported that *T. aripo* avoids leaves infested by *E. fustis* and vice versa. This avoidance behavior may also contribute to the reduction in frequency of encounters between *T. aripo* and native phytoseiids on the cassava plant.

We had expected, based on the laboratory avoidance studies by Gnanvossou et al. (2003a), a down ward shift in within-plant distribution of native phytoseiids such as *E. fustis* and *I. degenerans* in the presence of *T. aripo* on the cassava plant. We did not detect, however, any changes in within-plant distribution of the two native phytoseiids after *T. aripo* introduction. The absence of such changes might be real, or might be due to the timing of sample selection. All of our sampling was conducted during daylight hours when all *T. aripo* individuals are in the terminal apices of the plant. It is possible that a downward shift in within-plant distribution of native phytoseiids in Malawi would have been observed if we had selected our samples during the night hours when *T. aripo* is foraging on the upper 20% of the cassava foliage. If it is true, however, that the native phytoseiids shift their distribution downward during the night hours, the lack of changes in their within-plant distribution patterns—based on day-light hours observations—as a result of the introduction of *T. aripo* may also indicate that native phytoseiids adjust their within-plant distribution upward during the daylight hours after *T. aripo* had retreated to the terminal apices of the cassava plant. Verification of this hypothesis requires repeated day and night observations on within-plant distribution of the native phytoseiids in the presence and absence of *T. aripo*.

Our results show also that the introduction of *T. aripo* did not negatively affect the abundance of native phytoseiids inhabiting plants associated with cassava in Mozambique and Malawi. *T. aripo* populations now found in Africa are specific to cassava plants and are rarely found on other plants found in cassava fields (Yaninek and Hanna, 2003; Zannou et al., 2005b). The risks of competition for food and of intraguild predation between *T. aripo* and the native phytoseiids inhabiting associated plants are therefore limited. It is possible, however, that indirect negative effects could occur, particularly with *E. baetae* in Mozambique and *I. degenerans* in Malawi, as both phytoseiids occur on cassava and its associated vegetation.

While negative effects, in terms of the abundance of native phytoseiids, were not detected, there is evidence of a positive effect on the abundance of the two native phytoseiids

E. fustis and *I. degenerans* in Malawi, and a change in the within-plant distribution of *E. baetae*. That *E. fustis* and *I. degenerans* in Malawi were more abundant on cassava (both predators) and its associated vegetation (*I. degenerans* only on the latter) at 1 and 2 years of *T. aripo* presence compared with their abundance prior to *T. aripo* introduction may very well reflect facilitation of the two native phytoseiids by *T. aripo* introduction. A recent review of the literature indicates that facilitation of native species by the introduction of alien species is a common phenomenon (Rodriguez, 2006). In the system reported in this article, facilitation could be the result of an avoidance behavior of *M. tanajoa* to *T. aripo* movement out of the apex of the plant during the night hours, a behavior shown in two studies (Magalhaes et al., 2002; Onzo et al., 2003b). Movement of *M. tanajoa* downward on the cassava plant leads to greater probability of contact with *E. fustis* and *I. degenerans* which inhabit largely the middle and lower strata of the cassava canopy. This increase in food supply is likely to lead to an increase in the abundance of *E. fustis* and *I. degenerans* and may very well explain the facilitation phenomenon observed after *T. aripo* introduction in Malawi. There is abundant evidence that both *E. fustis* and *I. degenerans* feed and reproduce on *M. tanajoa* (e.g., Akpokodje et al., 1990; Bruce-Oliver et al., 1996; Nwilene and Nachman, 1996; Onzo et al., 2005) and it is quite likely that native phytoseiids complement *T. aripo* suppression of *M. tanajoa* populations in Malawi. It is not known, however, if this applies to the biological control of *M. tanajoa* in Mozambique, as we do not know to what extent *E. baetae*, the most abundant phytoseiid on cassava in Mozambique, feeds and reproduces on *M. tanajoa*.

Within-plant distribution of *E. baetae* was affected by the introduction of *T. aripo* as shown by the reduction in proportion of *E. baetae* on the top part of the cassava plant. This is likely due to avoidance of *T. aripo* by *E. baetae* and not intraguild predation by *T. aripo* on *E. baetae* (as average total per plant density of *E. baetae* would have been lower than expected under the avoidance only hypothesis). Migration of *E. baetae* to mature leaves is unlikely to have considerably affected *M. tanajoa* biocontrol as densities of the latter declined after 1 and 2 years of *T. aripo* presence in Mozambique (Hanna et al., unpublished data).

The absence of negative effects of *T. aripo* introduction and the documentation of facilitation on the most common native phytoseiids in cassava fields, while achieving successful biological control of the target pest, together contribute to making the campaign against of *M. tanajoa* in Africa a rare example of successful classical biological control with a phytoseiid predator and without any negative effect on the environment. Indeed, the success of the introduction of a natural enemy is measured by its capacity to reduce target pest density and to maintain it below economically damaging levels, without undesirable effect on non-target organisms of the agro-system where it is introduced (Symondson et al., 2002). This lack of negative effects on local phytoseiid fauna is particularly significant because the native phytoseiids also feed on *M. tanajoa* (Bruce-Oliver et al., 1996; Nwilene and Nachman, 1996; McMurtry and Croft, 1997; van Rijn and Tanigoshi, 1999) and, is effect, complement biological control of *M. tanajoa* by *T. aripo*. Should there have been a drastic reduction in native phytoseiid abundance, lower impact of *T. aripo* on *M. tanajoa* populations would have been likely observed; which is not the case anywhere *T. aripo* was introduced and established in sub-Saharan Africa (Hanna and Toko, 2003; Yaninek and Hanna, 2003; Hanna et al., 2005). Coexistence of native phytoseiids with *T. aripo* would then reinforce the control of *M. tanajoa*. Indeed, recent studies on the role of native and introduced phytoseiids in *M. tanajoa* suppression from Benin, West Africa (Onzo et al., 2003a) indicated that the predatory mite guild composed of *T. aripo* and native phytoseiids had a significant impact on the abundance of *M. tanajoa.* Similar observations were recorded in Malawi and Mozambique (Zannou et al., unpublished data).

Overall, this study provides consistent data across three years over an expanse of over 700,000 km^2 indicating the absence of negative effects of the introduction of *T. aripo* into southern Africa, as measured by changes in abundance and within-plant distribution of native phytoseiid predators found in cassava fields. One cannot, however, rule-out undesirable effects occurring over periods longer than observed during the present study. At least from a perspective of *M. tanajoa* biological control, subsequent surveys conducted in Malawi and Mozambique indicated that *T. aripo* has persisted (including areas surveyed by the present study) and that *M. tanajoa* densities continued to decline (Hanna et al., unpublished data). Socioeconomic benefits of *M. tanajoa* biocontrol in southern Africa has not been quantified, but preliminary calculations indicate that they are likely as large as the benefits of *M. tanajoa* biocontrol in western Africa (Hanna and Toko, 2003).

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