A major subtropical fruit pest accumulates in crop fields and spills over to a wild host

Courtney Moxley[a,b], Wiebke Lammers[c], F. J. Frank van Veen[c], Elisa Thébault[d], Karen J. Esler[b] and Colleen L. Seymour[a,e] *

[a]South African National Biodiversity Institute, Kirstenbosch Research Centre, Private Bag X7, Claremont, 7735, South Africa.

[b]Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa.

[c]Centre of Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Penryn Campus, Penryn, Cornwall, TR10 9FE, United Kingdom

[d]Institute of Ecology and Environmental Sciences – IEES Paris (UMR 7618, UPMC, CNRS, IRD, INRA, UPEC, Paris Diderot), Université Pierre et Marie Curie, Tour 44-45, 4 place Jussieu, Paris, France

[e]DST/NRF Centre of Excellence at the Percy FitzPatrick Institute of African Ornithology, Department of Biological Sciences, University of Cape Town, Rondebosch, 7701

*Corresponding author. Email:c.seymour@sanbi.org.za, Tel. +27 21 799 8856

Abstract

Wild plant species are often considered a source of crop pests in mixed landscapes but this view rarely considers pest spillover in the opposite direction (from crop fields to natural vegetation), or spatiotemporal variability in resources between crop and wild habitats. We investigate how infestation of mango crop (*Mangifera indica*, Anacardiaceae) and a related wild host (marula, *Sclerocarya birrea*, Anacardiaceae) by a major subtropical fruit crop pest (*Ceratitis cosyra*, Diptera: Tephritidae) varies with distance from the boundary between crop and natural vegetation. We determined how infestation of marula is associated with proximity to mango crops at field and landscape scales over two fruiting seasons on three farms in north-eastern South Africa. This is one of few studies to date to consider pest spillover from crop fields to natural vegetation and the only one performed in a biodiverse region with relatively little habitat transformation. Over three sampling periods, *C. cosyra* infestation of marula always decreased with distance from mango fields. At the landscape scale, marula alongside crop fields were 30 times more likely to be infested than in distant vegetation (1.3 – 6 km from mango), suggesting that spillover occurs from crop fields to natural vegetation. During late mango and marula fruiting, twice as many flies infested marula than mango. However, over the two months post-mango fruiting, up to 25 times more *C. cosyra* were trapped in mango fields than in bordering natural vegetation. Although pests spillover from crop fields into natural vegetation to use marula as an alternate host, biological control in the natural vegetation may eliminate this habitat as a pest

reservoir outside the crop season. Other nearby crops may be more important than wild hosts for maintaining *C. cosyra* out of mango season. Landscape planning should consider proximity and arrangement of fields containing crops that host shared pests at different times of the year.

Keywords: agroecology, pest control, polyphagous pests, population reservoir, related plant hosts, spatiotemporal variability

1. Introduction

Agriculture has fragmented natural ecosystems worldwide, leaving mixed landscapes with patches of natural vegetation interspersed among human-managed agroecosystems (Benton et al., 2003). Biological communities in these landscapes are spatially and temporally dynamic (Thies et al., 2005); if consumer species are supported by resources in both crop and non-crop patches, they may move freely between the two habitats (Tscharntke et al., 2005). This spillover has been widely studied, with much focus on its effect on ecosystem services and disservices in agroecosystems (Blitzer et al., 2012 and Rand et al., 2006). Many insect herbivores are crop pests responsible for large-scale production and economic losses in agricultural systems (Oerke, 2006), so understanding insect spillover informs how habitat transformation affects ecological functioning in these systems.

Studies on spillover have tended to focus on movement of pests from natural vegetation to crop fields because this research is of most interest to farmers. Over 100 studies reviewed by Norris and Kogan (2009) show that natural vegetation is a source of insect herbivores that shift into crop fields. In natural vegetation, host plants are dispersed, making them difficult for pests to locate, but crop monocultures provide a concentrated resource on which pests may accumulate in high densities (Root, 1973). Crops are only available at certain times of the year, however, and resource alteration after crop harvesting often forces pests onto alternate hosts in nearby natural vegetation (Altieri and Letourneau, 1982).

77 Spillover from crop fields to natural vegetation is far less understood; a
78 recent review by Blitzer et al. (2012) identified only three studies that
79 investigated spillover in this direction (Kaiser et al., 2008, Mckone et al.,
80 2001 and Squires et al., 2009). Nevertheless, spillover from crop fields to
81 natural vegetation is likely common given that many insect herbivores are
82 polyphagous habitat generalists (Tscharntke et al., 2005), with many
83 using both crops and wild plants as hosts (Norris and Kogan, 2009). Such
84 spillover suggests that wild hosts may act as a refuge for pests outside the
85 crop season (Mckone et al., 2001).

86 Opposing predictions have been made for whether insect pests shift from
87 habitats of low to high resource concentration (i.e. from natural
88 vegetation to crop) or vice versa, with evidence of pest spillover across
89 the crop-non-crop interface suggesting that natural vegetation can be
90 either a source or a sink (a population "reservoir" or secondary host
91 source) in mixed agricultural landscapes (reviewed by Tscharntke et al.,
92 2005). Cultural pest-control schemes often target wild hosts in natural
93 vegetation by managing or removing wild plants before the crop season,
94 without considering these conflicting findings and the broader
95 spatiotemporal dynamics of mixed agricultural landscapes (Herzog and
96 Funderburk, 1986). Removing alternate hosts in surrounding natural
97 vegetation can reduce crop-pest infestation by encouraging dispersal of
98 natural enemies into crop fields, promoting biological pest control (e.g.
99 Cottrell and Yeargan, 1999). However, some wild hosts may provide
100 crops with "associational resistance" to infestation by retaining pests in
101 natural vegetation (reviewed by Barbosa et al., 2009), where predation

rates can be higher (Henri et al., 2015). Removing these wild hosts may encourage pest spillover onto nearby crops.

A major pest of mango (*Mangifera indica* L.Anacardiaceae), the mango fruit fly, *Ceratitis cosyra* (Walker) (Diptera: Tephritidae), also uses marula fruits (*Sclerocarya birrea* (A. Rich) Hochst., Anacardiaceae) in nearby natural vegetation. *Ceratitis cosyra* is polyphagous, using 33 other crop and non-crop species as hosts throughout Sub-Saharan Africa (De Meyer et al., 2002). Mango and marula fruiting overlap between November and April and consequently, marula is considered an important reservoir for *C. cosyra* (Copeland et al., 2006). Marula is often managed by farmers at the start of mango season by spraying auxins to facilitate early marula ripening, followed by burial, incineration or removal of fruits from natural vegetation on farmlands.

Relative timing of the onset of mango and marula fruiting varies between years, likely because marula fruiting is more coupled to rainfall than mango, which receives irrigation throughout the year. This variation results in marula fruiting earlier than mango in some years but later in others. Given that the net direction of spillover depends on spatiotemporal dynamics in productivity between habitats (Ries et al., 2004), marula could be either a source or reservoir for *C. cosyra*, depending on relative resource availability between habitats between seasons.

We investigated fruit infestation in mango and marula at increasing distances from the habitat margin in crop fields and natural vegetation, respectively, at field and landscape scales. Since related host species

display increased susceptibility to infestation when they occur in close proximity (Barbosa et al., 2009), we expected that marula fruit infestation would be highest in natural vegetation nearest to mango fields. In three periods varying in relative marula-mango resource availability, we asked:

1. How is marula fruit infestation in natural vegetation associated with distance to nearby mango fields (field scale)?
2. How is marula infestation associated with proximity to mango fields at the landscape scale?
3. Does *C. cosyra* accumulate in mango fields or natural vegetation at the end of the crop fruiting season?

2. Methods and materials

2.1 Study site and species

The study was conducted on three mango farming estates (Bavaria Fruit Estates, Mohlatsi and Venden) in the Kruger to Canyons Biosphere Region, a biodiverse area of north-eastern South Africa, of which half is set aside for conservation (Coetzer et al., 2010). Mango are farmed in single cultivar blocks (~70 x 150 m) separated by a single row of *Casuarina* sp. trees serving as windbreaks. Other subtropical fruits, including several *Citrus* spp., passion fruit (*Passiflora edulis*) and avocado (*Persea americana*) are also cultivated on these farms over the year. Farms practise conventional pest control using chemical pesticides throughout the year.

The farms have patches of natural vegetation alongside some crop fields, with the habitats separated by a 10-25 m margin. The natural vegetation is "Granitic Lowveld" savanna, dominated by woody *Acacia* spp. and S. *birrea* (marula) (Mucina and Rutherford, 2006). Marula is dioecious; females bear small fruits (mass = ~20 g) with a large pit and soft, fleshy skin when ripening on the ground.

Ceratitis cosyra is endemic to sub-Saharan Africa where it can cause up to 73% losses to the annual mango crop in some countries (Vayssières et al., 2009). Adult flies damage fruits by ovipositing their eggs beneath the fruit skin, where larvae hatch and feed, later dropping to the soil to pupate and eclose as adults (Hill, 1983).

2.2 How is marula fruit infestation in natural vegetation associated with distance to nearby mango fields?

2.2.1 Fruit collection and processing

In natural vegetation alongside mango fields at the three farms we sampled fruit from randomly distributed marula trees (>40 m apart) at various distances (4-275 m) from the field-natural vegetation margin. From 15 trees at Bavaria and 10 trees at Mohlatsi and Venden, we collected up to 20 fruits from the ground surrounding each tree, wherever possible (range: 1 – 20 fruits per tree). We sampled in three periods differing in relative availability of marula and mango: 1) Late marula/ late mango fruiting (March 2014, n = 302 marula fruits); 2) Early marula/peak mango fruiting (January 2015, n = 304); 3) Late marula/post-mango (30 days later, February 2015, n = 605), when mango fruiting had ended and

crop fields had been cleared of all fruit. Bavaria was the only farm sampled in 2014, while all farms were sampled in both seasons in 2015.

Marula fruits were placed into individual polystyrene cups with ~4 cm depth dry, sterile sand as a substrate for fly pupation, covered with chiffon secured with an elastic band to prevent emerged adult flies from escaping while permitting air flow. Cups were stored at ambient temperature (~27 ºC) for at least 35 days before adult flies were counted and identified as *C. cosyra*. Larvae and pupae that failed to develop and eclose were considered dead due to parasitism or other causes and could not be identified to species. These were assumed to be *C. cosyra* and included in total fly count per fruit.

2.2.2 Data analysis

We ran two separate generalized linear mixed effects models (GLMMs) to investigate how distance from the habitat margin is associated with 1) the likelihood of marula infestation by *C. cosyra* and 2) infestation intensity. GLMMs are suitable for analysing non-normal data that are pseudo-replicated in space and time (Bolker et al., 2009).

The likelihood of marula infestation was investigated using a binomial GLMM (with logit function) and *C. cosyra* presence/absence per fruit as the response variable. Infestation intensity was investigated using a GLMM with negative binomial error structure (with log function) and *C. cosyra* abundance per fruit as the response variable. This error structure accounts for overdispersion without the need to transform the count data (Zuur et al. 2009). We included a zero-inflated parameter in the model to account for the excess zeros in the count data

196 (R package: glmmADMB, Bolker et al. 2012) and to reduce the risk of Type I
197 errors (Martin et al. 2015).

198 Both models included distance from the habitat margin and sample season
199 as fixed effects. Tree and farm were included as random effects to
200 account for pseudoreplication, repeat sampling of trees between seasons
201 and non-independence of fruits sampled from the same tree.

202 2.3 How is marula infestation associated with proximity to
203 mango fields at the landscape scale?

204 2.3.1 Fruit collection and processing

205 To determine how marula infestation is associated with proximity to
206 mango at the landscape scale during (January 2015) and after mango
207 fruiting (February 2015), we collected marula in both periods from five
208 trees (>40 m apart) at distances of 4-275 m from the margin along two
209 parallel transects (~100 m apart) at each of Bavaria, Mohlatsi and Venden
210 Estates. In distant vegetation (1.3-6 km), we sampled five randomly-
211 distributed trees (>35 m apart) in each of two conservation areas with
212 relatively little human disturbance, Hoedspruit Wildlife Estate and
213 Raptor's View.

214 Marula availability and ripeness may influence *Ceratiti*s population size
215 and distribution (Sciarretta and Trematerra, 2011). Therefore, we
216 recorded total fruit abundance within canopies and on the ground
217 surrounding each tree, and the number of fruiting marula trees within a 20
218 m radius of each tree. We also collected 10 fruit within three ripeness
219 categories per tree where possible: 1) "unfallen, unripe": green fruit

220 within the canopy, 2) “fallen, unripe” and 3) “fallen, ripe”: green-white
221 and yellow fruit on the ground surrounding each tree, respectively.

222 Fruit were stored in polystyrene cups for 28-31 days before emerged flies
223 were counted and identified as *C. cosyra*. Pupae that failed to eclose were
224 assumed to be *C. cosyra* and included in the total fly count per fruit.

225 2.3.2 Data analysis

226 We used separate binomial and zero-truncated, negative binomial
227 GLMMs to determine how *C. cosyra* presence/absence and abundance
228 per fruit, respectively, differ alongside mango fields and in the distant
229 vegetation (Jan: $n = 675$, Feb: $n = 833$). Both models included a binary
230 factor for proximity to mango fields (nearby/distant), sample season, fruit
231 ripeness, the abundance of fruit in/surrounding the tree and the number of
232 surrounding trees as fixed effects, and tree nested within site (including
233 farms and distant conservation areas) as random effects.

234 2.4 Does *C. cosyra* accumulate in mango fields or natural
235 vegetation at the end of the fruiting season?

236 2.4.1 Fruit collection and processing

237 In March 2014, we collected between 2 – 10 mangoes (Kent cultivar,
238 mass = ~600 g) (depending on fruit availability) from trees at varying
239 distances from the natural vegetation along four parallel transect (>100 m
240 apart) in fields on Bavaria Fruit Estate: 0 m ($n = 18$ mangoes), 10 m ($n =$
241 21), 50 m ($n = 19$), 100 m ($n = 21$), 200 m ($n = 16$) and 500 m ($n = 13$).

These transects were mirrored in the natural vegetation opposite each field, with five marula trees (>40 m apart) sampled at 8-370 m from the margin along each transect. Twenty marula fruits were collected from the ground around each tree where possible (range: 1-20 fruit per tree) (n = 399).

Mangoes were placed into separate perforated, plastic bags with a portion of sand for 2-3 weeks. Emerged larvae and pupae were placed into individual polystyrene cups containing sand and a piece of damp tissue to retain moisture for pupation. Cups were covered with perforated plastic film and stored for 2 weeks before eclosed adult flies were counted and identified to species. Two other species also infest mango in addition to *C. cosyra*: *C. rosa* and *C. capitata*. These two species are not known to use marula as an alternative host and were excluded from analyses.

Marula were placed into individual polystyrene cups as above and stored for at least 35 days before emerged adult flies were counted and identified. All flies were identified as *C. cosyra*, and dead larvae/pupae were assumed to be *C. cosyra* and added to the total fly count per fruit.

2.4.2 Pheromone trapping

We used pheromone traps (Sensus^{TM} traps, River Bioscience, Port Elizabeth, South Africa) to determine where *C. cosyra* accumulate after mango and marula fruiting has ended. Trapping was conducted in both mango fields and natural vegetation on Bavaria Fruit Estate, along four parallel transects (~100 m) at 0, 10, 50, 100, 200 m from the field-natural vegetation margin in four 2-week periods (early April, late April, early

May, late May 2014, i.e. 160 traps in total) when fruits were no longer available in either habitat. Traps were equipped with Questlure bait for female *Ceratitis* flies (Insect Science Ltd, Tzaneen, South Africa) and a Dichlorovos tablet, which was replaced after 4 weeks to maintain trapping efficiency. Flies were counted and identified after 11 – 14 days in each sample period.

2.4.3 Data analysis

For fly emergence data, we used binomial and negative binomial GLMMs to compare *C. cosyra* presence/absence and abundance per fruit, respectively, between mango and marula. The negative binomial model included a parameter to account for zero-inflation of the count data. Both models included fruit type and distance from the habitat margin as fixed effects, and tree nested within transect as random effects to account for pseudoreplication and non-independence of fruits from the same tree.

For pheromone trap data, we used a negative binomial GLMM to compare *C. cosyra* abundance between habitats and over several periods after mango/marula fruiting has ended. We used fly abundance per trap as the response variable, and habitat, distance from the habitat margin and sample period as fixed effects. Trap nested within transect were random effects to account for pseudoreplication and repeat trapping at the same locations between sample periods (Bates, 2016).

2.5 Model selection

All analyses were performed using R (R Core Team, 2014), with GLMMs fitted for random intercept analyses using packages lme4 (Bates, 2016) and glmmADMB. Before fitting the models, we checked for outliers and collinearity using pairwise scatterplots of explanatory variables (Dormann et al., 2013). For each count model, we considered Poisson and negative binomial error structure and compared model fit using Akaike Information Criteria (AIC). We also compared GLMMs structured for random intercept and random slopes using AIC (Zuur et al., 2009). In all cases, negative binomial models and random intercept GLMMs had the lowest AIC values and were considered better models. For model validation, residuals were plotted against fitted values and explanatory variables to check for overdispersion. Log-likelihood ratio tests and AIC were used to identify optimal models in backward model simplification. For each GLMM, we determined the proportion of variance explained by fixed and random effects (conditional R^2) and fixed effects only (marginal R^2) using (Nakagawa and Schielzeth, 2013).

3. Results

3.1 How is marula fruit infestation in natural vegetation associated with distance to nearby mango fields?

At the field scale, distance from mango had no effect on the likelihood of infestation per marula ($\chi^2_1 = 2.46$, $p = 0.12$), regardless of sample period ($\chi^2_1 = 1.03$, $p = 0.60$). However, intensity of infestation decreased with distance from mango ($\chi^2_1 = 5.00$, $p = 0.025$, Fig. 1), with average fly

abundance per fruit declining by ~37% (~2 flies) from 4 m to 275 m into the natural vegetation.

Marula were ~2-3 times more likely to be infested when early marula fruiting coincided with peak mango fruiting than at the end of mango fruiting or when mango had been cleared ($\chi^2_1 = 32.21$, $p < 0.001$, Fig. 2) (R^2 marginal = 0.34; R^2 conditional = 0.46). Approximately twice as many flies emerged per marula fruit on average in early marula/peak mango (1.45 ± 0.20) and late marula/post-mango fruiting periods (5.46 ± 0.49) than when late marula and mango fruiting coincided (3.12 ± 0.27) ($\chi^2_1 = 48.82$, $p < 0.001$) (R^2 marginal = 0.47; R^2 conditional = 0.86).

3.2 How is marula infestation associated with proximity to mango fields at the landscape scale?

Marula were ~30 times more likely on average to be infested alongside mango fields than in the distant vegetation ($\chi^2_1 = 13.20$, $p < 0.001$). The distance effect did not vary with sample period ($\chi^2_1 = 0.07$, $p = 0.79$), although marula were almost twice as likely to be infested before than after mango harvesting ($\chi^2_1 = 6.67$, $p < 0.01$) (R^2 marginal = 0.72; R^2 conditional = 0.78). Although the likelihood of being infested was markedly different between near and distant sites, once infested, fruit fly abundance per fruit did not differ alongside mango fields (11.78 ± 0.25) or in distant vegetation (8.00 ± 0.25; $\chi^2_1 = 1.15$, $p = 0.56$), regardless of sample period ($\chi^2_1 = 1.54$, $p = 0.46$). However, marula infestation was affected by fruit ripeness and fruit abundance in the tree canopy and on the ground surrounding the tree (Fig. A.1).

3.3 Does *C. cosyra* accumulate in mango fields or natural vegetation at the end of the fruiting season?

Fly emergence from fruit

Adult flies reared from mango were *Ceratitis cosyra* (92%), *C. rosa* (5%) and *C. capitata* (3%). Dead larvae/pupae comprised 70% and 10% of the total emerged individuals from mango and marula, respectively. The proportion of emerged individuals that failed to develop was not associated with distance from the margin in both fruit types ($\chi^2_1 = 1.11$, $p = 0.29$; Table A.1).

Since we cannot distinguish the three *Ceratitis* species among larvae/pupae emerging from mango, we first ran GLMMs with data including only adult *C. cosyra* flies from mango (and total *C. cosyra* abundance from marula). Fruit infestation was not associated with distance from the margin in either mango or marula (infestation likelihood: $\chi^2_1 > 0.01$, $p = 0.99$; infestation intensity: $\chi^2_1 = 0.231$, $p = 0.64$). However, marula were ~4 times more likely to be infested ($\chi^2_1 = 14.28$, $p < 0.001$) and had ~3 times more flies emerging per fruit than mango ($\chi^2_1 = 7.46$, $p < 0.01$).

We also ran GLMMs with data including both adult *C. cosyra* flies and a proportion of dead larvae/pupae emerging from mango (and total *C. cosyra* abundance from marula). This was based on the assumption that there was equal likelihood of mortality for all three *Ceratitis* species, so we included 92% of larvae/pupae from mango as *C. cosyra* in the total fly count per fruit.

Plotted residuals of these GLMMs indicated an outlying data point (51 flies from mango at 50 m from margin) in the fruit infestation data containing a proportion of larvae/pupae from mango. This point exerted excessive influence on the model parameter estimation (function influencePlot:car, Fox 2015). We excluded it from the data (n = 506) and re-analysed the data. Results including the outlier are presented in Table A.2.

With the outlier removed, the likelihood of infestation was equal between mango and marula (χ^2_1 = 1.55, p = 0.46) and was not associated with distance from the habitat margin (χ^2_1 = 0.09, p = 0.76). However, marula had twice as many flies on average emerging per gram of marula (0.08 ± 0.009 flies/g) than mango (0.002 ± 0.0005 flies/g) (z = 2.57, df = 2, p = 0.01).

Fly abundance per mango increased with distance from natural vegetation and decreased with distance from mango fields in marula (χ^2_2 = 3.95, p = 0.047, Fig. 3) (R^2 marginal = 0.51; R^2 conditional = 0.59). The increase in infestation intensity with distance in mango fields (slope = 0.0027x) was twice as strong as the decrease with distance in natural vegetation (-0.0012x).

Pheromone traps

For two months after mango and marula fruiting, *C. cosyra* abundance in pheromone traps was not associated with distance in either habitat (χ^2_1 = 2.81, p = 0.42).

382 Differences in *C. cosyra* abundance trapped in mango fields and natural
383 vegetation varied with time since mango and marula fruiting ended (χ^2_4 =
384 12.8, $p < 0.01$, Fig. 4). Immediately after fruiting had ended (early April),
385 ~25 times as many *C. cosyra* flies on average were collected per trap in
386 mango fields (5.60 ± 1.96) than in natural vegetation (0.20 ± 0.09). The
387 abundance of flies trapped in both habitats increased significantly in late
388 April, with only 3 times as many flies on average collected per trap in
389 mango fields (6.95 ± 2.16) than in natural vegetation (2.10 ± 0.57). In
390 early and late May, 6.5 and 20 times more flies were collected per trap on
391 average in mango fields (1.85 ± 0.46; 0.9 ± 0.30) than in natural
392 vegetation (0.35 ± 0.13; 0.05 ± 0.05), respectively (R^2 marginal = 0.80;
393 R^2 conditional = 0.87).

394 ## 4. Discussion

395 We found that a polyphagous pest, *Ceratitis cosyra*, spills-over from crop
396 fields into natural vegetation in search of alternate wild hosts at the end of
397 crop fruiting. The pest remained in crop fields for two months after crop
398 and wild host fruiting has ended, suggesting that mango fields are more
399 likely to be a pest reservoir than natural vegetation at the end of mango
400 fruiting. Our results also point to the importance of differences in peak
401 fruit timing between crop and natural hosts, which influenced both the
402 likelihood and intensity of pest infestation. Below, we discuss the
403 implications of these findings for management of pests using both crop
404 and wild hosts in a mixed landscape.

405

4.1 How is marula fruit infestation in natural vegetation associated with distance to nearby mango fields?

Decreasing abundance of *C. cosyra* per marula fruit with distance from mango fields suggests that the pest spills-over from crop fields into nearby natural vegetation. This occurred both when mango fruiting had ended or continued over marula season. Gradients in resource availability at the landscape scale drive pest dispersal between habitats differing in resource concentration in mixed landscapes (Tscharntke et al., 2005). Considering that monoculture crops provide a concentrated resource that frequently support high pest abundance (Marques et al., 2000), contrasting with the low-density and randomly-distributed resources available in natural vegetation, dispersal of *C. cosyra* from crop fields into natural vegetation may be expected.

Flies may also be driven out into nearby natural vegetation when pesticides are applied in crop fields before and during the harvest. The farms practice conventional pest control using insecticides, such as neonicotinoids or organophosphates, which they spray in low concentration throughout the year, including during the mango fruiting season (November – March). In sampled fields on Bavaria Fruit Estates, pesticides are sprayed every month except for a period at the end of and after mango fruiting season (March – May). Absence of pesticide during these months may account for the accumulation of *C. cosyra* in crop

fields for two months after mango/marula fruiting season (See section 4.3).

Adult female flies may not only disperse out of crop fields in search of alternate hosts, but also for resources like food and shelter, which are removed from crop fields by mowing in-crop weeds in corridors between rows of mango trees. Mowing occurs after mango harvesting (April – June) as another form of pest control on the farms.

4.2 How is marula infestation associated with proximity to mango fields at the landscape scale?

Although *Ceratitis* adults can disperse up to 50 km in some instances (Israely et al., 2005), they are generally poor dispersers, remaining within tens of metres but up to 400-700 m from the point of emergence from hosts (Meats and Smallridge, 2007). This would explain the marked difference in infestation probability of marula (30 times greater) near mango fields than in distant vegetation, both when mango was available or had been cleared from crop fields, and provides further evidence of spillover from crop to natural vegetation. Accumulation of *C. cosyra* alongside mango fields may suggest that the pest encounters substantial alternate host resources here without having to disperse over large distances into distant vegetation.

By acting as an alternate host for *C. cosyra* when mango is fruiting, marula may act as a "decoy" that retains the pest (Holmes and Barrett, 1997) and provides the crop with "associational resistance" to infestation. Previous research in our study area showed higher predation rates of

Ceratitis larvae in natural vegetation than in mango fields (Henri et al., 2015). Therefore, marula removal may actually drive pests into crop fields but this requires further research. Nevertheless, the success of area-wide pest management programs that target wild hosts has not been widely documented to date (Norris and Kogan, 2009).

4.3 Does *C. cosyra* accumulate in mango fields or natural vegetation at the end of the fruiting season?

We consider it more reasonable to consider a proportion of larvae/pupae in the total *C. cosyra* count from mango than to include only *C. cosyra* adults in the analyses. Firstly, we reared adults from only 9 mango, while 34 mangos had *Ceratitis* larvae, pupae and adults emerge. This high larval/pupal mortality is likely due individuals drowning in large volumes of liquid as the mangos (~600 g) rotted. Owing to the low emergence success of adult *Ceratitis* for mango, the results from the adult model alone are an underestimate of mango infestation. Secondly, population dynamics among *Ceratitis* species appear to cyclical, with *C. cosyra* able to sustain its populations for longer at the end of the fruit season than the other two species (W. Lammers, unpublished data). Since *C. cosyra* was the dominant adult emerging from mango, it is reasonable to assume that the same proportion of larvae/pupae would also be *C. cosyra*. Below, we discuss results from the analyses including these individuals.

Marula is an important alternate host for *C. cosyra* when the mango resource becomes less abundant, given that marula were more severely infested than mango in the late mango/marula fruiting season. During this

477 season, the pest appears to spillover from crop fields and disperse evenly
478 through both habitats in search of hosts, as suggested by equal infestation
479 likelihood between fruit types and the absence of distance effects in either
480 habitat.

481 Greater infestation intensity per marula may have resulted from easier
482 host detection in the natural vegetation, where fallen fruits remain on the
483 ground surrounding trees (Fig. A.1), compared to crop fields, where fallen
484 fruits are removed as part of sanitation practices. Alternatively, higher
485 pest loads on marula may be linked to harsh conditions in crop fields,
486 including the use of chemical pesticides and mowing in-crop weeds,
487 which might drive *C. cosyra* into nearby natural vegetation in search of
488 alternate hosts, shelter and food (See Section 4.1).

489 Host abundance in the landscape also influences pest spill-over between
490 habitats (Tscharntke et al. 2005). Using 2013 mango production data from
491 Bavaria Fruit Estates and recorded marula fruit abundance from this
492 study, we extrapolated the total number of flies/g of fruit to a hectare of
493 Kent mango fields and adjacent natural vegetation. A hectare of mango
494 containing 1200 ± 1100 kg of fruit (mean ± SD) (~500 trees) would yield
495 2400 ± 2150 flies, while a hectare of natural vegetation containing 180 kg
496 marula (15 trees, randomly dispersed) would yield 980 ± 700 flies. This
497 estimate is based on average flies emerging per fruit from a single season
498 (late mango/marula fruiting, March 2014) but the infestation rate between
499 the fruits may change as the relative availability of the fruits changes (See
500 Section 4.5 below).

The estimate should be considered in context of an agricultural landscape, with isolated patches of natural vegetation interspersed amongst hundreds of hectares of crop fields. In this context, the crop fields on large farming estates provide a larger, more concentrated host resource for *C. cosyra*, despite higher infestation per fruit among marula observed in this study, resulting in large-scale spillover and higher infestation of the dispersed marula trees in nearby natural vegetation.

Indeed, this is supported by consistently greater fly abundance trapped in mango fields over two months after mango and marula fruiting has ended. This also provides further evidence against natural vegetation as a reservoir for *C. cosyra* outside of mango fruiting. Around this time (May – July), winter crops that are known to host *C. cosyra*, such as several *Citrus* spp., avocado (*Persea americana*) and passion fruit (*Passiflora edulis*) (De Meyer et al., 2002) begin fruiting on farms sampled here. Unlike other climate-sensitive *Ceratitis* species that are dormant over the winter in South Africa (e.g. *Ceratitis capitata*; *C. rosa*), *C. cosyra* persists on available hosts throughout the year (De Villiers et al., 2013). Therefore, by harbouring higher relative *C. cosyra* abundance long after marula/mango season, mango fields themselves may provide an important source of pests for winter crops. Thus, crops may be more important for *C. cosyra* population cycling throughout the year than wild hosts such as marula in the natural vegetation.

Despite its polyphagous nature, *C. cosyra* was not reared from fruit samples from 121 species of non-crop plants in natural vegetation, including known hosts of the species (e.g. *Cucurbita*, *Ficus*, *Opuntia*,

Solanum and *Strychnos* spp., (De Meyer et al., 2002), throughout winter (April – July 2014; C. Moxley, unpublished data), suggesting that the pest is not hosted at significant densities in the natural vegetation. Further research into landscape-scale population cycling could benefit conservationists and advocates of natural vegetation in agricultural landscapes by elucidating the relative importance of other wild hosts and crops in harbouring pests.

4.4 Natural pest control in natural vegetation

Flies trapped after mango and marula fruiting had likely emerged after pupating in the soil over the two months since dropping from fruit. Lower abundance per trap in natural vegetation may be explained by greater predation of *Ceratitis* pupae in natural vegetation compared to mango fields (Henri et al., 2015).

During our rearing experiment, nine Opiinae parasitoid wasps emerged from marula, while none emerged from mango. This is likely an under-estimate of parasitoid density because we limited further parasitism by removing fruit from the field and parasitoids take longer to emerge, beyond the time we allowed for *C. cosyra* (C. Moxley, unpublished data). While higher larval/pupal mortality in mango (70%) than marula (10%) may suggest that parasitism by natural enemies is greater in crop fields, we observed no association between the proportion of eclosed individuals in both mango and marula and the distance from the margin (Table A.1). Causes for the high larval/pupal mortality outlined above may also explain the lack of parasitoid wasps emerging from mango.

550 Distance effects detected here provide additional evidence for stronger
551 pest control in natural vegetation than mango fields. In mango fields, flies
552 may encounter more natural enemies along margins nearest natural
553 vegetation where pesticides are less concentrated, and weedy borders
554 provide habitats and complementary resources for natural enemies. Our
555 findings are consistent with those of Henri et al., 2015, where adult
556 *Ceratitis* abundance increased and pupal predation decreased in mango
557 fields with distance from natural vegetation, suggesting that natural
558 enemies are favoured over pest populations in natural vegetation, as
559 predicted elsewhere (Chaplin-Kramer et al., 2011).

560 Data in this section were collected on a single estate (~2 km) and in one
561 fruiting season (March 2014). At present, we cannot determine whether
562 the weak distance effects are driven by other gradients along the sampled
563 edge. To test the generality of our findings, mango/marula infestation
564 should be considered across multiple, spatially independent edges on
565 several farms in the future.

566 **4.5 Effects of seasonal differences in relative marula-mango**
567 **resource availability**

568 Spatiotemporal shifts in resource availability drive spillover of consumer
569 species, including insect pests, between habitats in mixed agricultural
570 landscapes (Gavriel et al., 2012). Here, marula infestation by *C. cosyra*
571 seemed to change with relative availability of the mango crop. Marula
572 had the greatest infestation when early marula fruiting coincided with
573 peak crop fruiting, suggesting spillover of *C. cosyra* from crop fields.

574 Interestingly, when marula was the only host resource available for *C.*
575 *cosyra* in the landscape (after mango fruiting), marula had lower
576 infestation than when mango was in peak fruiting, providing further
577 evidence against natural vegetation as a population reservoir for *C. cosyra*
578 at the end of crop fruiting. Rather, *C. cosyra* appeared to shift to marula
579 as a secondary resource, in contrast to the concentrated resource provided
580 by the crop.

581 These conclusions are derived from data collected in only two seasons.
582 Further long-term studies are needed to establish how infestation of the
583 wild host, and its role in harbouring the pest depends on timing of crop
584 fruiting relative to the wild host, as well as availability of other crops in
585 the landscape. This could inform optimal spatial configuration of crop
586 fields in the landscape.

587 ## 4.6 Implications for conservation

588 While negative impacts of pest spill-over from natural vegetation into
589 crop fields can be quantified economically as declines in crop production,
590 it is less straightforward to assess impacts of pest spillover in the reverse
591 direction. Effects may be indirect, such as decreased ecosystem
592 functioning, resulting from competition for shared resources (e.g. food
593 and shelter) and consequent displacement of pollinators and natural
594 enemies by the pest (e.g. Pearce, 2001). The pest may also associate with
595 other wild plants besides marula in the natural vegetation (See Section
596 4.3) and displace native herbivores from their hosts. This may also impact
597 the reproductive success of these plants (e.g. Kaiser et al. 2008) by

affecting seed health or the likelihood of fruits being eaten and dispersed. Such effects may be of particular concern at close proximity to crop fields at the field scale, rather than the landscape scale, if the pest is generally a weak disperser like *Ceratitis* (Meats & Smallridge 2007). Future extensions of this work to consider such impacts on marula would benefit conservation, given that marula is a keystone species in the lowveld savanna (Shackleton et al. 2002).

6. Conclusions

Our data suggest that the primary direction of pest spillover is from crop fields into nearby natural vegetation. Wild hosts in distant vegetation (1.3 – 6.2 km away from crop fields) are less likely to support the pests than natural vegetation in close proximity to the crop host. Natural vegetation does not necessarily harbour the pest outside the crop season, even if the pest accumulates on the wild host at the end of fruiting, possibly because of biological pest control in natural vegetation. It is likely that the nature of *C. cosyra*'s lifecycle, in which it pupates in the soil over two months, facilitates its transition to other crops that begin to fruit one to two months after mango fruiting has ended. Thus, the wild host may only act as a temporary alternate host at the end of mango fruiting, and greater likelihood of predation in natural vegetation may reduce pest numbers.

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Figure Legends

Fig. 1. Fly abundance per marula fruit with distance from the margin into natural vegetation (data from N = 3 farms). Lines and equations represent negative binomial glm fits of mean values ± 1 SE. Point size is weighted by frequency of fruit with the same number of flies emerging per distance point. The y-axis is truncated to exclude five data points for visual clarity (40, 46 and 54 flies per fruit at 8 m from the margin, 36 at 40 m and 55 flies at 275 m) but all data were included in analyses.

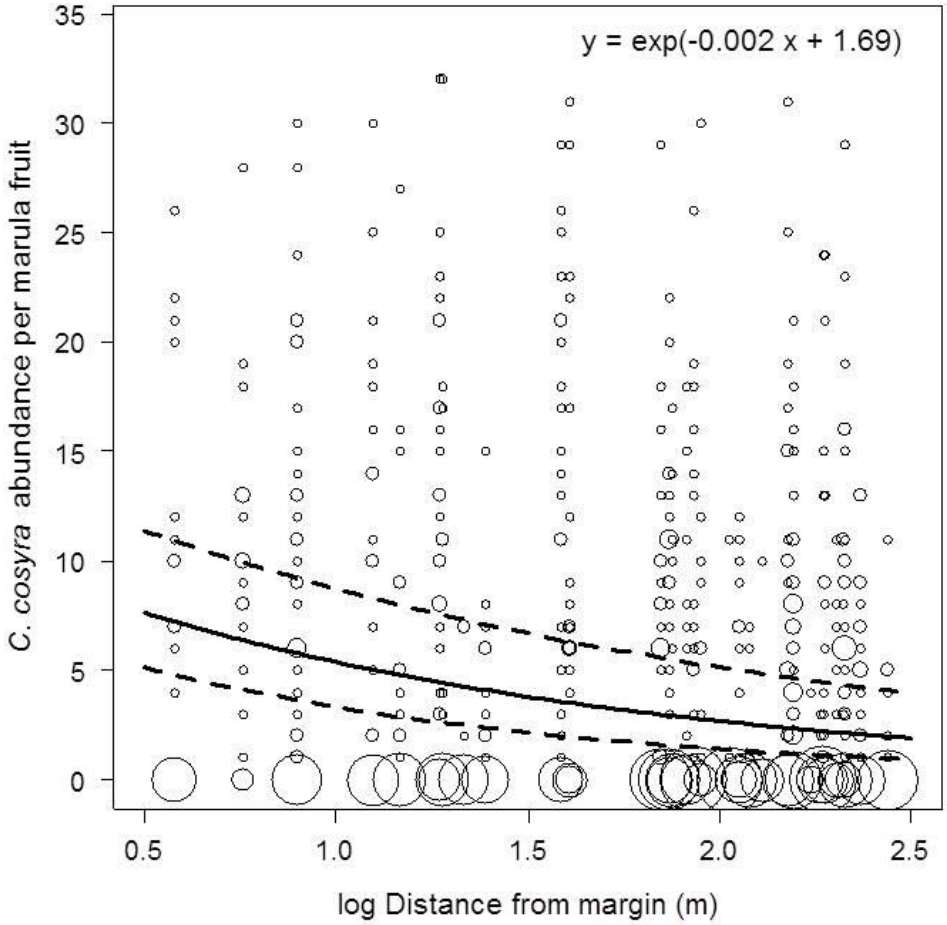
Fig. 2. Fly abundance per marula fruit in three periods differing in relative marula/mango resource availability (data from N = 3 farms): 1) Late/Late ’14: late marula fruiting coincides with late mango (2014), 2) Early/Peak ’15: early marula fruiting coincides with peak mango fruiting (2015) and 3) Late/Post ’15: late marula fruiting coincides with post mango fruiting (2015). Letters denote differences in infestation intensity per fruit between sample periods, with significance at $\alpha = 0.05$. The y-axis is truncated to exclude five data points for visual clarity (46 and 54 flies per fruit in early season 2015, and 36, 40 and 55 in late season 2015) but all data were included in analyses.

Fig. 3. Fly abundance per fruit at increasing distance from the habitat margin into (a) natural vegetation and (b) mango fields (data from N = 1 farm). Lines and equation represent model of best fit ± 1 SE. Point size is weighted by frequency of fruit with the same number of flies emerging per distance point. Figure does not include an outlier (51 flies emerging at 50 m from margin) from mango fields and trends are derived from model that excluded the outlier.

Fig. 4. Fly abundance per Sensus^{TM} trap in mango fields and natural vegetation in early and late April and May 2014 (data from N = 1 farm). Letters denote differences in abundance between habitats and periods, with significance at α

796 = 0.05 determined from negative binomial GLMM. The y-axis is truncated to
797 exclude three outliers >20 flies per trap for visual clarity.
798

799 **Figure 1**



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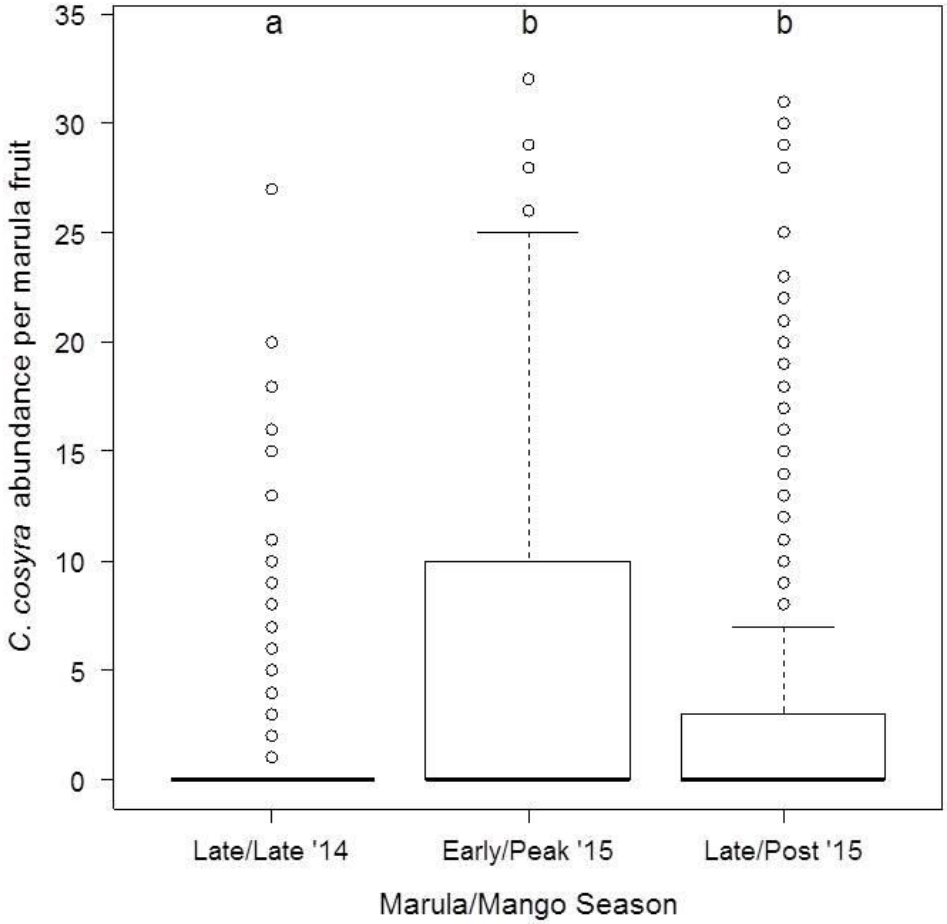
802 **Figure 2**



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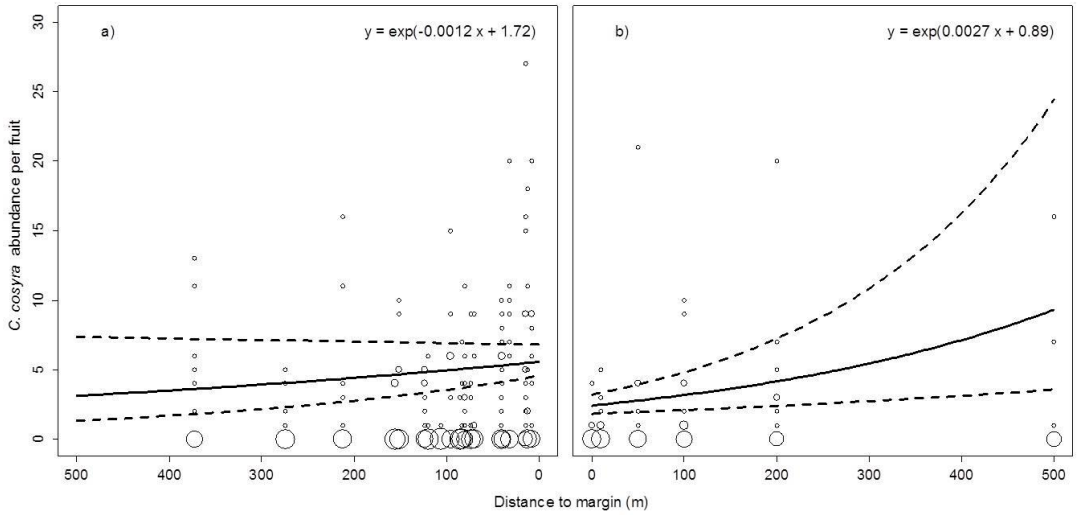

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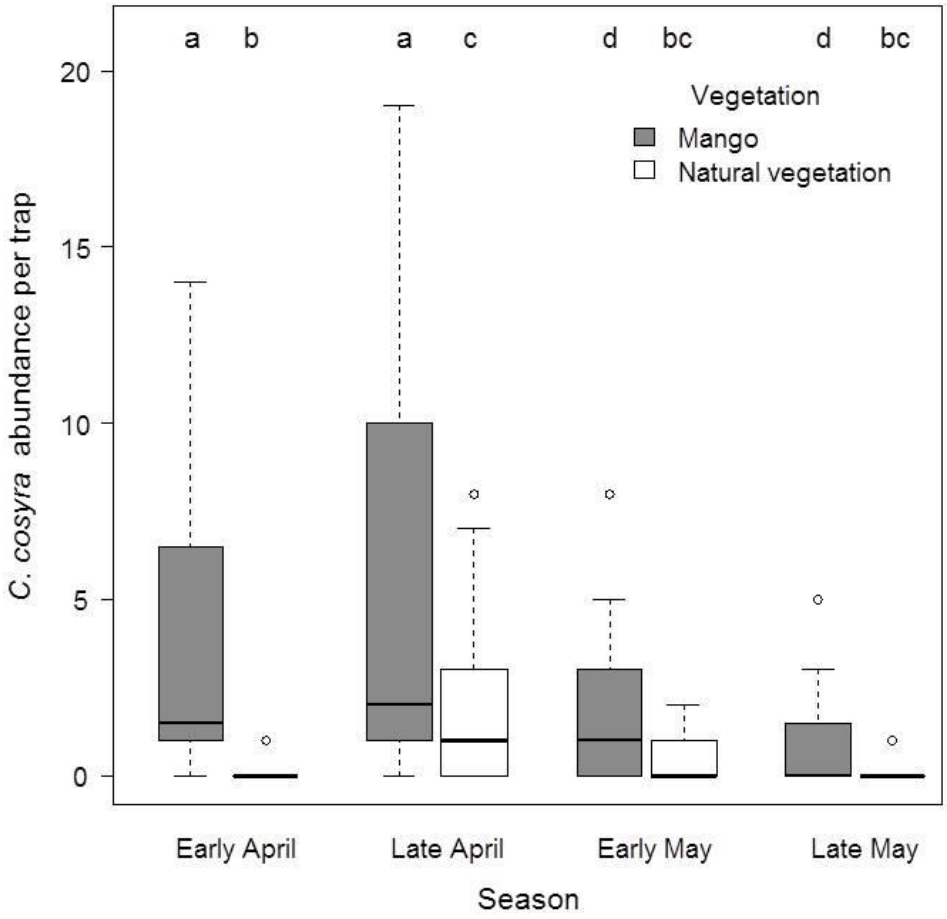
808 **Figure 4**



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