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Original article

Fruit flies associated with the pepper-bark tree (*Warburgia salutaris* (G.Bertol.) Chiov.) in Mpumalanga province, South Africa

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Summary

Introduction – **The pepper-bark tree, *Warburgia salutaris* (G.Bertol.) Chiov. (Canellaceae) is used in southern Africa as a medicinal plant and plays an important role in traditional healing systems. Over-harvesting, indigenous timber clearing, agriculture and human living requirements all threaten the tree's future existence in the wild and therefore, is categorized as an endangered tree (A1acd ver 2.3) on the IUCN Red List. Fruit are often heavily infested with fruit fly larvae (Diptera: Tephritidae), which damage the seed. Three *Ceratitis* spp. and one *Zeugodacus* sp. were documented to infest fruit of *Warburgia* spp. in Africa. No studies have been carried out in South Africa. The aim of the study was to identify fruit fly species infesting the fruit in Mpumalanga province of South Africa.** *Materials and methods* – **Fruit were sampled during 2014–2017 at three locations in Mpumalanga province. Fruit were randomly sampled from July to November and include samples from the tree and ground. Fruit flies were also trapped in the vicinity of *W. salutaris* trees at Skukuza indigenous nursery, Kruger National Park. Fruit flies were identified by using a multi-entry key. The number of fruit flies per kg fruit was determined for each sample.** *Results and discussion* – **A total of 7,427 Marula fruit fly, *Ceratitis cosyra* (Walker) (Diptera: Tephritidae) emerged from the sampled fruit and no other species were found. Fruit throughout the fruiting period were affected. The infestation index for *C. cosyra* varied between 398.5 to 2,851.7 adult fruit flies per kg fruit. Parasitic wasps of fruit flies belonging to the families Braconidae and Eulophidae also emerged from the fruit. During 2016, 68% of fruit were infested while in 2017, 69% were infested when randomly sampled from the ground at White River. *C. cosyra* was the abundant species trapped.** *Conclusion* – **When fruit are collected for seed extraction and propagation, it is important to do fruit fly trapping and fruit inspection. Fruit can be covered with bags or sleeves for protection against *C. cosyra*. Bait stations and sanitation can also be used for suppression of fruit fly numbers.**

Keywords

Ceratitis, *Ceratitis cosyra*, Marula fruit fly, Tephritidae

Significance of this study

What is already known on this subject?

- The pepper-bark tree, *Warburgia salutaris* (G.Bertol.) Chiov. (Canellaceae) is widely used in southern Africa as a medicinal plant and plays an important role in traditional healing systems.
- The tree is an endangered plant species.
- Fruit are often heavily infested with fruit fly larvae (Diptera: Tephritidae), which damage the seed.
- No previous studies were conducted in South Africa to identify fruit flies associated with the fruit of *W. salutaris.*

What are the new findings?

- A total of 7,427 Marula fruit fly, *Ceratitis cosyra* (Walker) (Diptera: Tephritidae) emerged from the sampled fruit.
- The infestation index was high and varied between 398.5 to 2,851.7 adult fruit flies per kg fruit.
- During 2016, 68% of fruit were infested while in 2017, 69% were infested as sampled from the ground at White River.

What is the expected impact on horticulture?

- *W. salutaris* can be propagated from seed by managing *C. cosyra*.

Introduction

Warburgia Engl. is a genus in the Canellaceae Martius family occurring in Africa (Maroyi, 2014; Leonard and Viljoen, 2015). The pepper-bark tree, *Warburgia salutaris* (G.Bertol.) Chiov., is the only species of *Warburgia* that is found in southern Africa and is present in Lesotho, South Africa, Swaziland, Malawi, Mozambique and Zimbabwe (Coates Palgrave, 2002; Van Wyk *et al.*, 2009; Maroyi, 2013). The tree is evergreen, slender and grows up to 10 m tall, but can reach a height of 20 m (Coates Palgrave, 2002). The fruit are oval berries, narrowed towards the base, green, turning dark purple when ripe, leathery in texture and covered with glands (Figure 1). The plant contains several drimane and colorotane sesquiterpenoides which are responsible for a variety of biological activities including antibacterial, antifungal, antifeedant, cytotoxic, phytotoxic, piscicidal, and molluscicidal (Maroyi, 2013, 2014; Leonard and Viljoen, 2015). The tree is used as a traditional medicine of various cultural groups in South Africa (Maroyi, 2013). The bark, leaves and roots are widely used to treat numerous health complaints such as coughs,

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Figure 1. *Warburgia salutaris* fruit.

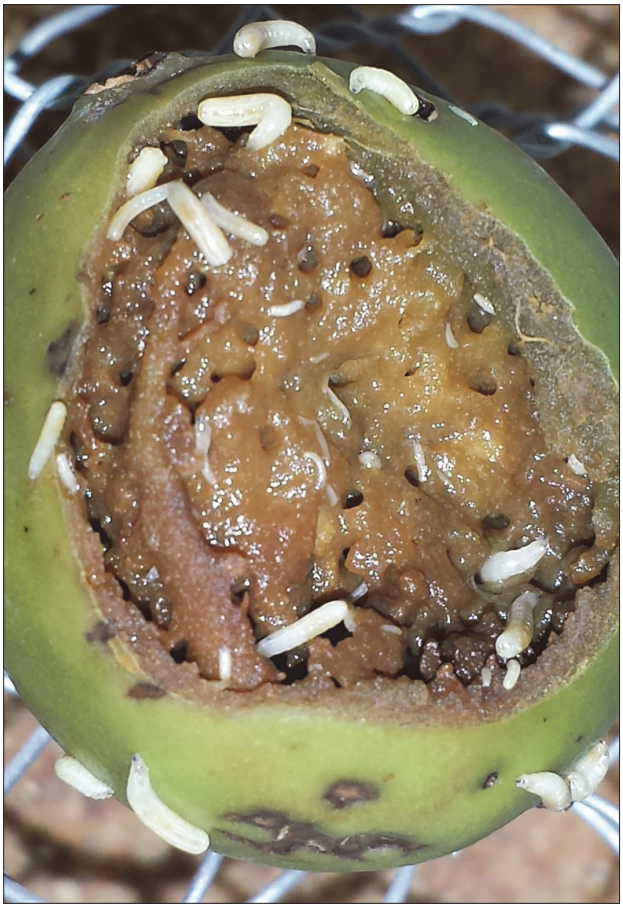

Figure 2. *Warburgia salutaris* fruit heavily infested with fruit fly larvae.

colds, chest complaints, influenza, rheumatism, malaria, venereal diseases, headache, toothache and gastric ulcers (Van Wyk *et al*., 2009; Maroyi, 2013; Leonard and Viljoen, 2015). Overharvesting, indigenous timber clearing, agriculture and human living requirements all threaten the tree's future existence in the wild. There is a decline in the population and therefore *W. salutaris* is categorized as endangered (A1acd ver 2.3) on the IUCN Red List (Hilton-Taylor *et al*., 1998). Various projects are investigating methods of propagation under controlled conditions, with subsequent supply of trees to traditional healers and gene bank establishments (Hannweg *et al*., 2016). *W. salutaris* is cultivated from seed and vegetatively from cuttings. Fruit are often heavily infested with fruit fly larvae (Diptera: Tephritidae) (Figure 2). Fruit fly larval infestations damage the seed, reducing the viability (Xaba and McVay, 2010).

In Africa, there are about 400 species of frugivorous fruit flies (Diptera: Tephritidae) (Virgilio *et al*., 2014). More than 50 of them are of economic significance. The female punctures the fruit with her ovipositor and deposits eggs within the host fruit. Larval development is completed within the fruit, which as a result may become rotten. Fruit flies documented to infest the fruit of *Warburgia* spp. included *Ceratitis contramedia* (Munro), the Marula fruit fly, *Ceratitis cosyra* (Walker), *Ceratitis fasciventris* (Bezzi) and the melon fruit fly, *Zeugodacus cucurbitae* (Coquillett) (De Meyer and White, 2004; De Meyer *et al*., 2015b). *C. contramedia* is only known in Kenya and South Africa. *C. contramedia* was recorded from *Carissa macrocarpa* Eckl. A.DC. (original record *Carissa grandiflora* (E.Mey.) A.DC.) (Apocynaceae) in South Africa (De Meyer, 1998; De Meyer and White, 2004). *C. cosyra* is widely distributed in sub-Saharan Africa and is a well-documented pest of mango and other cultivated crops (Vayssières *et al*.,

Table 1. Sampling of *Warburgia salutaris* fruit.

Sample no.	Sampling site (tree or ground)	Date	No. Fruit	Weight (g)	No. of *C. cosyra*	No. of Hymenoptera parasitoids	Infestation index (no. of *C. cosyra* per kg fruit)	No. of parasitoids per kg fruit
1	Skukuza (tree)	5 Jul. 14	–	52.7	21	7 Braconidae	398.5	132.8 Braconidae
2	Skukuza (tree)	10 Sep. 14	29	66.3	76	0	1,145.6	
3	Mbombela (tree)	14 Sep. 14	1	–	46	0	–	
4	Skukuza (tree)	10 Oct. 14	33	88.7	253	14 Braconidae 6 Eulophidae	2,851.7	157.8 Braconidae 67.6 Eulophidae
5	Skukuza (tree)	23 Oct. 14	19	70.7	98	7 Braconidae	1,386.9	99.0 Braconidae
6	Skukuza (tree)	21 Aug. 15	33	95.3	200	0	2,098.6	
7	Skukuza (tree)	23 Sep. 15	47	203.1	316	8 Braconidae	1,555.9	39.4 Braconidae
8	Skukuza (tree)	15 Oct. 15	31	156.9	133	0	847.7	
9	Skukuza (tree)	12 Nov. 15	59	308.3	286	0	927.9	
10	White River (ground)	23 Aug. 16	310	2,784.3	4,775	571 Braconidae 19 Eulophidae	1,715.0	205.1 Braconide 6.8 Eulophidae
11	White River (ground)	18 Aug. 17	144	2,280.0	1,223	372 Braconidae	536.4	163.2 Braconidae

Mean infestation index (± Standard deviation) = 1,346.4 (± 747.7).

2014; Rwomushana and Tanga, 2016), and is especially associated with fruits of the Marula tree, *Sclerocarya birrea* (A. Rich) Hochst. (Anacardiaceae) (Grové *et al*., 2017). *C. fasciventris* is found throughout western Africa, with isolated records from Central Africa, and extensive distribution along the Albertine and Gregory Rifts in eastern Africa, as far north as Ethiopia and is of economic significance (De Meyer *et al*., 2015a). *Z. cucurbitae* is an invasive species in Africa but not recorded in South Africa (De Meyer *et al*., 2015b).

W. salutaris fruit samples collected in Maputo province, Mozambique were all infested with *C. cosyra* (Muatinte and Cugala, 2014). A higher fruit fly incidence was present in fruit collected from the ground compared with fruit sampled from the tree. It was speculated that the fruit fly infestation might cause premature fruit drop. No studies were conducted in South Africa to identify fruit fly species associated with *W. salutaris* fruit.

The objective of this study was to identify the fruit fly species responsible for damage to the *W. salutaris* fruit in the Mpumalanga province, South Africa. This information is important for the development of a management strategy in order to propagate *W. salutaris* from seed.

Materials and methods

Fruit were sampled at three locations in Mpumalanga province:

1. Skukuza indigenous nursery, Kruger National Park (lat. -24.984545, long. 31.579055, alt. 279 m). Climate: BSh (semi-arid climate or steppe climate) according to the Köppen-Geiger climate classification (Kottek *et al*., 2006). Vegetation type: Granite Lowveld (SVI 3), Savanna Biome (Mucina and Rutherford, 2006). Fruit were sampled from one tree planted in 1996 at the Skukuza nursery. There were two more *W. salutaris* trees in the nearby vicinity, but these trees had little fruit. Numerous indigenous tree species were growing at and in the vicinity the nursery. Fruit were randomly sampled four times during the fruiting period in 2014 (samples no. 1 and 3–5) and 2015 (samples no. 6–10) (Table 1).
2. The experimental farm of the Agricultural Research Council, Tropical and Subtropical Crops, City of Mbombela (lat. -25.461351, long. 30.972246, alt. 719 m). Climate: Cwa (Monsoon-influenced humid subtropical climate) according to the Köppen-Geiger climate classification (Kottek *et al*., 2006). Vegetation type: Legogote Sour Bushveld (SVI 9), Savanna Biome (Mucina and Rutherford, 2006). One sample was taken from a tree growing on the experimental farm during 2014 (sample no. 2) (Table 1).
3. Small holding, White River (lat. -25.332703, long. 31.041089, alt. 937 m) Climate: Cwa (Monsoon-influenced humid subtropical climate) according to the Köppen-Geiger climate classification (Kottek *et al*., 2006). Vegetation type: Legogote Sour Bushveld (SVI 9), Savanna Biome (Mucina and Rutherford, 2006). The site at White River consisted of *W. salutaris* trees growing in a garden on a smallholding outside the town. Various fruits were cultivated on a small scale on the holding. Samples no. 10 and 11 were taken from the ground over two consecutive years (2016–2017) and fruit were collected randomly from the ground from the group of trees.

Therefore, the sample size and the frequency of sampling differed between the three locations and depended on the availability of fruit. From the three sites, more than 562 fruit were sampled (sample 1 – number of fruit not counted) and the weight of the samples was 2.296 kg (sample 2 – no weight). The *W. salutaris* trees in these above-mentioned areas usually start flowering in March and the first fruit was found in July/August. The fruiting period usually ends about October/November.

Collected fruit were taken to the laboratory and counted and weighed. Fruit were placed on chicken wire mesh in 5-L cylindrical plastic containers containing sterilized sand. The sand served as the pupation medium for the larvae. Mesh-fitted lids were placed on the containers to ensure enough ventilation. Fruit were held at room temperature for approximately eight weeks. Puparia were left in the containers to emerge. Emerged adults and parasitoids were collected. The infestation index was calculated as the number of emerged adult flies per 1 kg of fruit (Cowley *et al*., 1992). During 2016 and 2017, the percentage fruit that was infested with fruit flies was determined. Therefore, one hundred fruit were sampled from the ground at White River in 2016 and 2017 and dissected and inspected for infestation.

Fruit flies were trapped at Skukuza nursery from 17 August 2015 to 14 December 2015 to determine which species were present in the vicinity of the *W. salutaris* trees when fruiting. A Chempac yellow bucket trap (Chempac (Pty) Ltd., Suider Paarl, South Africa) with Biolure® Fruit Fly (Chempac (Pty) Ltd.) was placed in a *W. salutaris* tree. Biolure® Fruit Fly is a food bait that consists of three components, *i.e.*, ammonium acetate, trimethylamine hydrochloride and 1,4-diaminobutane (putrescine). Both males and females of various fruit fly genera are attracted to the lure. A block that contains 195 g kg^{-1} dichlorvos (Vapona agricultural insecticide strips) (Acorn Products (Pty) Ltd., Strubens Valley, South Africa) was placed in the trap as a killing agent. Lures and blocks were replaced every six weeks. The trap was serviced on a weekly basis and collected flies were taken to the laboratory where flies were identified and the sex determined. The number of fruit flies per trap per day was calculated for the abundant species.

A multi-entry key was used for the identification of emerged and trapped fruit flies (Virgilio *et al.*, 2014). Emerged fruit flies were also sent to the Royal Museum for Central Africa, Tervuren, Belgium for confirmation of identification by M. De Meyer. Parasitoids were identified to family level with a key for parasitoids of fruit-infesting Tephritidae (Wharton and Yoder, 2020). The number of parasitoids found was expressed as the number per kg fruit.

Results

Fruit flies emerged from all four samples taken at Skukuza in each of the two years, 2014 and 2015 (Table 1). Fruit flies were also found in the one sample collected at Mbombela and the two samples collected at White River. A total number of 7,427 fruit flies emerged from the 11 samples taken (Table 1). *C. cosyra* was the only fruit fly species found from the fruit. The infestation index varied between 398.5 to 2,851.7 *C. cosyra* per kg fruit with a mean of 1,346.4 (Table 1). The mean infestation index (±SD) for the samples taken at Skukuza nursery from the trees for 2014 and 2015 were 1,445.7 (±773.8) and 1,357.5 (±586.9) *C. cosyra* per kg fruit, respectively. Therefore, a 6% decrease in the mean infestation index was present from 2014 to 2015. The sample with the highest infestation index during 2014 was sampled at 10 October 2014. There was a 187% increase in the infestation index from the first to the second sample and a 149% increase from the second to the third sample. Afterwards, there was a 51% decrease from the third to the forth sample. During 2015 the sample with the highest infestation index was 21 August 2015. There was a 26% decrease from the first to the second sample and a 46% decrease from the second to the third sample. An increase of 9% was present from the third to the forth sample. Fruit sampled from the ground at White River had an infestation index of 1,715.0 and 536.4 *C. cosyra* per kg fruit during 2016 and 2017, respectively. Therefore, a decrease of 69% in the infestation index was present from 2016 to 2017. In 2016, 68% of the 100 fruit sampled from the ground at White River were infested, while in 2017, 69% were infested. In infested fruit, seeds were found to be totally destroyed by the larval activity or holes were observed in the seeds. Only uninfested fruit had intact seed.

Parasitic wasps of fruit flies belonging to the families Braconidae and Eulophidae emerged from the fruit. During 2014, three samples contained parasitoids at Skukuza nursery (Table 1). One sample contained both Braconidae and Eulophidae while the other two contained only Braconidae. In 2015, only one contained Braconidae parasitoids. Both samples taken at White River contained parasitoids. During 2016, both Braconidae and Eulophidae parasitoids were found but in 2017, only Baraconidae parasitoids were present. The number of Braconidae parasitoids per kg fruit varied between 39.0 and 201.1. The number of Eulophidae parasitoids per kg fruit in the two samples was 67.6 at Skukuza and 6.8 at White River.

Fruit flies trapped from17 August 2015 to 14 December 2015 were the invasive Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (no. trapped = 1); the Mediterranean fruit fly, *Ceratitis capitat*a (Wiedemann) (6); *C. cosyra* (206); *Ceratitis discussa* (Munro) (2); and the Natal fruit fly, *Ceratitis rosa sensu lato* Karsch (12). Therefore, 90.7% of fruit flies trapped were *C. cosyra*. More females of *C. cosyra* were trapped (females = 68%; males = 32%). The number of *C. cosyra* per trap per day is presented in Figure 3. Peak numbers were present in August 2015 and afterwards declined. The peak numbers in August 2015 correspond with the highest infestation index that was sampled in August 2015.

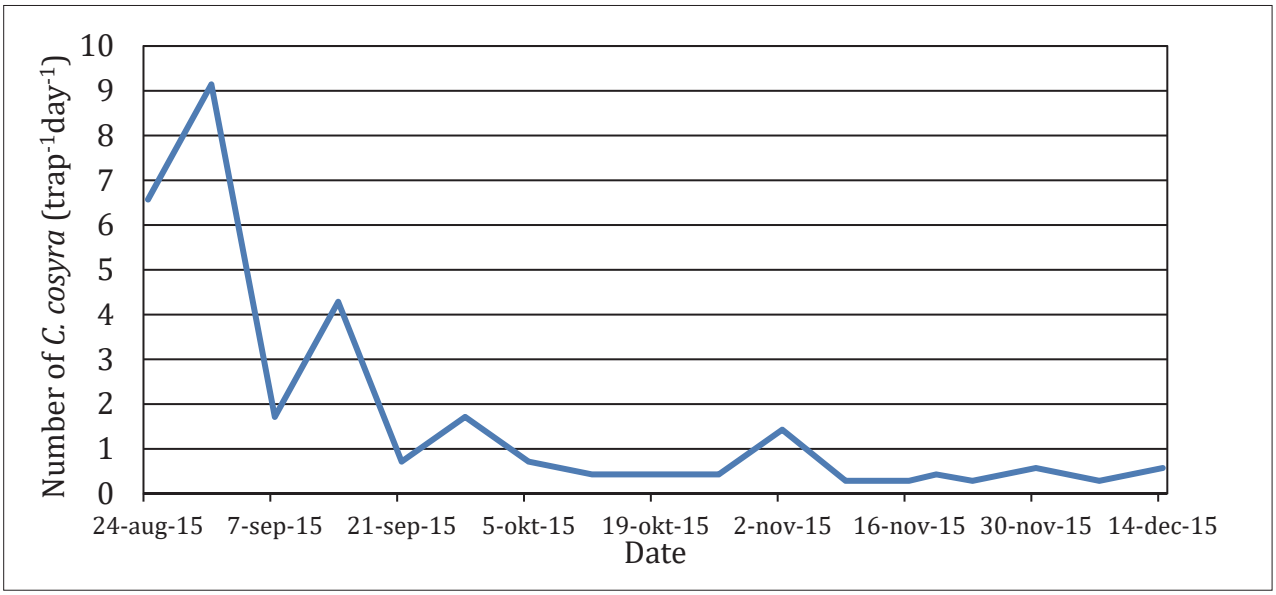

Figure 3. Number of *C. cosyra* trapped at Skukuza nursery with a bucket trap and Biolure® Fruit Fly.

Discussion

All samples collected in this study were infested irrespective of location (Table 1). The samples were collected at two different climatic zones according to the Köppen-Geiger climate classification. A high infestation index was found at both zones. Fruit flies were present in fruit sampled from the trees and from the ground. Fruit sampled from the beginning to the end of the fruiting period were infested at Skukuza. *C. cosyra* was the only fruit fly species found from the fruit and was responsible for damage caused to the *W. salutaris* fruit. In infested fruit it was observed that the larval activity destroyed the seed or made holes in the seed. *C. cosyra* was also the only species found from *W. salutaris* fruit in Mozambique (Muatinte and Cugala, 2014). Therefore, both studies had similar findings. Of the trapped species, *C. capitata*, *C. cosyra* and *C. rosa s.l.* and *B. dorsalis* are important pests for the production of cultivated fruits and also attack a wide range of indigenous fruits (De Meyer and White, 2004; Grové *et al*., 2017). *C. cosyra* has a more restricted host range compared with *C. capitata* and *C. rosa. B. dorsalis* is known to attack a wide range of host plants. The trapped species, *C. discussa* is known to attack fruit of the Annonaceae (De Meyer and White, 2004). A very high infestation index of *C. cosyra* was found and a high percentage of fruit was infested. Various studies were conducted in Africa with the aim of identifying host plants and infestation indices for fruit fly species (Copeland *et al*., 2006; Mwatawala *et al*. 2009; Badii *et al*., 2015; Grové *et al*., 2017, 2019). The infestation indices rarely exceeded more than 300 fruit flies per kg fruit in the latter studies. The infestation index in this study was therefore higher than that which occurred in *S. birrea* fruit, which is an important host plant for *C. cosyra* (Grové *et al*., 2017).

In this study, natural enemies were present belonging to the families Braconidae and Eulophidae which contributed to the biological control of *C. cosyra*. Brachonidae parasitoids of the genera *Fopius*, *Diachasmimorpha* and *Psyttallia* are known to be associated with *C. cosyra* in South Africa (Mohammed *et al*., 2016). These are all koinobiont solitary endophagous larval or egg-larval parasitoids. Eulophidae parasitoids associated with *C. cosyra* in South Africa belong to the genus *Tetrastichus* and are koinobiont gregarious endophagous parasitoids (Mohammed *et al*., 2016). Fruit fly populations are naturally controlled by co-evolved parasitic Hymenoptera. Studies showed that relatively smaller fruits generally seem to allow better control of infesting fruit flies by parasitoids than larger ones (Mohammed *et al*., 2016; Haran *et al*., 2019).

Various factors play a role in the process of leading a female fruit fly to a host plant. Plant odors that consist of a mixture of volatile compounds can play an important role in selection of suitable sites for oviposition (Quilicii *et al*., 2014). In the case of *W. salutaris,* it is not known what draws *C. cosyra* to the plant.

When *W. salutaris* fruit are collected to extract seed, it is important to protect the fruit from *C. cosyra* infestation. Trapping systems can be used to give an indication of *C. cosyra* numbers present in the vicinity of *W. salutaris* trees. Effective attractants for *C. cosyra* are 3-component lure (food based attractant) and enriched ginger oil (male attractant) which can be used with McPhail type traps (Grové *et al*., 2009; Joubert *et al*., 2015; Manrakhan *et al*., 2017). By cutting and visually inspecting the fruit, the presence of fruit fly larvae can also be determined. Fruit can be covered with paper bags or sleeves to prevent the fruit flies laying eggs in the fruit. It would be important to cover the fruit after fruit set. Sanitation can be used to suppress fruit fly numbers. The study in Mozambique showed the fruit on the ground had a higher fruit fly incidence compared with fruit on the tree (Muatinte and Cugala, 2014). Dropped fruit under the trees can be collected and removed to avoid re-infestation. Collected fruit can be placed in an augmentorium (a tent-like structure that sequesters fruit flies that emerge from the fruit but allow the escape of natural enemies through a fine mesh at the top of the tent). When trees are very tall and fruit difficult to reach, using bait stations could be an alternative method to suppress fruit fly numbers (Ekesi, 2016). Trees planted for seed extraction can be pruned to maintain size and make managing the fruit fly problem easier.

Conclusion

W. salutaris is an important medicinal plant and an endangered species. *C. cosyra* was found to heavily infest the fruit and damaged the seed in Mpumalanga, South Africa. When fruit are collected for seed extraction and propagation it is important to protect the fruit from fruit fly infestation. Trapping fruit flies and fruit dissection could give an indication of the presence of these pests. Recommended methods of control include fruit bagging, sanitation (removal of dropped fruit on the ground) and the use of bait stations. Parasitoids were found to contribute to the biological control of *C. cosyra* in the fruit. Future studies should investigate suppression methods for *C. cosyra* affecting *W. salutaris* which is environmentally-friendly, effective and affordable for local communities.

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