Seasonal Flight Patterns of Curculionidae (Cossoninae and Scolytinae) Infesting Dying *Euphorbia ingens* in South Africa[1]

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J. Entomol. Sci. 53(1): 70–81 (January 2018)

Abstract There is limited knowledge regarding the biology of beetles in Cossoninae and Scolytinae (Curculionidae) in South Africa. It has recently been found that beetles in these two weevil subfamilies are associated with *Euphorbia ingens* E. Meyer: Boissier die-offs in the country. Lindgren traps baited with 95% ethanol were set at three sites in two provinces of South Africa to gain an understanding of the seasonal activity of the beetles that infest *E. ingens*. Temperature and relative humidity were monitored at each site to correlate environmental conditions with beetle flight patterns. Seven beetle species, of which six were in Scolytinae and one in Cossoninae, were captured in the traps over a period of 20 mo. *Eccoptopterus spinosus* Olivier, *Premnobius cavipennis* Eichhoff and *Xyleborinus spinifer* Eggers were the most commonly caught beetles. *Ambrosiodmus natalensis* Schedl, *E. spinosus*, *P. cavipennis*, and *X. spinifer* are reported from South Africa for the first time. Of the seven beetle species, two, *Cyrtogenius africus* Wood and a *Stenoscelis* sp. Wollaston, are known to colonize diseased and dying *E. ingens* trees, but these were trapped in low numbers, possibly due to the choice of bait used. The number of *C. africus* and *Stenoscelis* sp. caught varied with temperature and humidity, but only temperature had a significant effect on numbers captured. The number of *C. africus* and *Stenoscelis* sp. caught appeared to be a function of site and climatic conditions as opposed to *E. ingens* mortality levels.

Key Words ambrosia beetles, *Cyrtogenius africus*, Lindgren funnel trap, weevils

Cossoninae and Scolytinae are subfamilies of the Curculionidae (Wood 1973). The Scolytinae are known as bark and ambrosia beetles, infesting woody plants (Bright 1976, Marvaldi 1997, Wood 1982). The incidence of infestation on seemingly healthy trees by Cossoninae and Scolytinae has increased over the last 20 yr and has been attributed to their increased introductions into novel environments (primarily due to a rise in global trade) and climate change (Liebhold et al. 1995, Pautasso et al. 2012, Pawson et al. 2013). Well-known examples include the exotic redbay ambrosia beetle *Xyleborus glabratus* Eichhoff, an invasive, and its fungal symbiont *Raffaelea lauricola* Harrington & Fraedrich (Fraedrich et al. 2008), which causes laurel wilt disease in native Lauraceae in the United States, and the extensive infestation of *Pinus contorta* Douglas (lodgepole

[1]Received 03 March 2017; accepted for publication 03 June 2017.

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pine) in British Columbia by the native mountain pine beetle *Dendroctonus ponderosae* Hopkins that is attributed to chronic warming and drought (Bentz et al. 2010, Cudmore et al. 2010, Konkin and Hopkins 2009).

Beetles in Cossoninae and Scolytinae have recently been collected from diseased *Euphorbia ingens* E. Meyer: Boissier in the KwaZulu Natal, Limpopo, Mpumalanga, and North West Provinces of South Africa (Roux et al. 2009, Van der Linde et al. 2011a, 2011b, 2001c, 2016, 2017). *Euphorbia ingens* is a succulent tree native to Africa occurring from South Africa to Kenya and Uganda, as well as Botswana, Malawi, Mozambique, Zambia and Zimbabwe (Gildenhuys 2006, Palgrave et al. 2002, Schmidt et al. 2002, Van Wyk and Van Wyk 1997). During the course of the last 15 yr, large-scale die-offs of *E. ingens* have been observed in South Africa (Malan 2006, Roux et al. 2008, 2009). Initial investigations of dying trees in the Limpopo Province reported the presence of *Cyrtogenius africus* Wood and *Euwallacea piceus* Motsch on *E. ingens* (Roux et al. 2009). Later studies also identified *C. africus* (Scolytinae), a *Cossonus* sp. Clairv (Cossoninae) and a *Stenoscelis* sp. Wollaston (Cossoninae) from dying *E. ingens* (Van der Linde et al. 2011a, 2001b, 2001c, 2016, 2017).

Significant differences in climatic conditions (temperature and precipitation) between areas with different levels of *E. ingens* mortality were observed in the Limpopo Province and North West Province of South Africa (Van der Linde et al. 2012). Such differences in environmental conditions could affect beetle activity and the timing and degree of attack on *E. ingens*. However, nothing is known regarding the flight activity of Cossoninae and Scolytinae in these two provinces. It is also not known whether differences in climatic conditions between the two provinces affect beetle flight timing and activity. The objectives of this study were, therefore, to monitor the seasonal flight patterns of Cossoninae and Scolytinae that infest *E. ingens* in areas with different *E. ingens* die-off levels and climatic conditions.

Materials and Methods

Study sites and collection of beetles. Cossoninae and Scolytinae were collected at three sites: two in the Limpopo Province (Last Post Game Ranch: GPS S 23°17′ 21.39″ E 29°55′ 27.93″, Capricorn: GPS S 23°21′ 50.67″ E 29°44′ 40.27″) and one in the North West Province (Enzelsberg: GPS S 25°22′ 58.05″ E 26°16′ 4.21″) of South Africa. Last Post and Enzelsberg are two privately owned game farms, whereas Capricorn is a privately owned production cattle farm. Sites in the Limpopo Province and North West Province had different levels of *E. ingens* die-off as well as different climatic conditions (Van der Linde et al. 2012). Selection of trapping sites was based on previous studies by Van der Linde et al. (2012) who described different levels of *E. ingens* die-off and infestation at the sites. The two sites in the Limpopo Province had the most significant climatic changes (increased temperature and variable rainfall patterns) over the last 50 yr (Van der Linde et al. 2012), with Enzelsberg being the most severely affected site, followed by Last Post and Capricorn (Van der Linde et al. 2017).

The traps used in this study were 11-unit black Lindgren funnel traps (Lindgren 1983), typically used for Scolytinae, that were suspended at a height of 1.5 m above ground level from a metal frame. Traps were baited with 95% ethanol (Reding et al.

2010) by using a 300-ml squeeze bottle containing 285 ml 100% ethanol and 15 ml distilled water with a 6.0-mm rope wick. Ethanol was used as a lure, because stressed trees are known to release this volatile organic compound that is used as a cue by many ambrosia beetles to locate suitable hosts (Kimmerer and Kozlowski 1982, Ranger et al. 2010). The wick was suspended into the liquid in the bottle, secured with a cable tie at the top with 5.0 cm of one end exposed to the air. Each lure was attached halfway along the length of the Lindgren trap by using cable ties. Ethanol was topped up biweekly during visits to collect beetles from traps. Ethylene glycol (Wynn's antifreeze/coolant, Wynn's Oil Company, Irwindale, CA) was placed in the collection cups, which were also refilled on collection days, with the collected beetles placed into 70% ethanol and transported to the laboratory for identification and quantification. Three traps were placed along a linear transect at each site within *E. ingens* stands, with a minimum of 50 m between each of the traps. The traps were placed in the field at the beginning of September 2013 (early spring) and removed at the end of April 2015 (midautumn). Beetles were divided into morphological groups (morphogroups) based on distinguishable characters (antennae, shape of the eye, elytral declivity, scutellum, and protibia) and then identified to species by Dr. Roger Beaver (161/2 Mu 5, Soi Wat Pranon, Chiangmai 50180, Thailand).

Temperature and relative humidity. Data loggers (Maxim iButton DS1923, Fairbridge Technologies, Johannesburg, South Africa) were suspended from one trap at each site to measure temperature and relative humidity every hour from 01 September 2013 through 30 April 2015. The data were downloaded with ColdChain ThermoDynamics software version 4.9 (Fairbridge Technologies, Sandton, Gauteng) and exported to Microsoft Excel for data analyses.

Statistical analyses. The mean monthly numbers captured for each beetle morphogroup were compared among each of the sites investigated. Normality of the data was tested using Shapiro–Wilk's test. Data that failed to meet the assumptions of normality were transformed ($\ln + 1$). If normality was not achieved after transformation, the data were analyzed with Kruskal–Wallis one-way analysis of variance (ANOVA). ANOVA was applied to raw data that met the assumptions of normality, and Student's *t*-tests were used when only two beetle morphogroups were compared. The mean temperature and relative humidity for each of the 20 mo during which collections were conducted were compared among the three sites by using ANOVA. Significant F and H tests were subjected to mean separation tests by using Tukey–Kramer's honest significant difference test ($P \leq 0.05$). Linear regression analyses were conducted to test whether the number of beetles caught was correlated with humidity and temperature or both. All statistical analyses were run using JMP Version 12.0.1 (SAS Institute 2015).

Results

Study sites and collection of beetles. Beetles caught at the three sites (Capricorn, Enzelsberg, Last Post) could be divided into seven morphogroups. Six were Scolytinae and one in Cossoninae. The Scolytinae were identified as *Ambrosiodmus natalensis* Schedl, *C. africus*, *Eccoptopterus spinosus* Olivier, *Premnobius cavipennis* Eichhoff, *Xyleborinus spinifer* Eggers, and *Xyloctonus latus*

Eggers, and the one Cossoninae as *Stenoscelis* sp. (Table 1). Two of the beetle species, *C. africus* and a *Stenoscelis* sp., are known to infest diseased *E. ingens* trees (Roux et al. 2009, Van der Linde et al. 2011a, 2011b, 2016, 2017).

There was a significant difference in the total number of beetle species captured among the sites ($H = 84.42$, df $= 6$, $P < 0.001$). The greatest number of total beetles caught was at Last Post (Table 1). The most commonly trapped beetle was *E. spinosus* (Table 1), although it was collected only at sites in the Limpopo Province and not at Enzelsberg in the North West Province. Very low numbers of *A. natalensis*, *C. africus*, *Stenoscelis* sp., and *X. latus* (*X. latus* was not caught at Capricorn) were collected. The most commonly caught beetle at Enzelsberg and Capricorn was *P. cavipennis*, whereas at Last Post, *E. spinosus* was the most commonly collected (Table 1). Except for *X. latus*, there was a significant difference in numbers of *A. natalensis*, *C. africus*, *E. spinosus*, *P. cavipennis*, *X. spinifer*, and *X. latus* caught among the sites (Table 1).

Cyrtogenius africus was caught in the highest numbers at the two sites in the Limpopo Province, whereas *Stenoscelis* sp. was caught in highest numbers at Enzelsberg (Table 1). There was a significant difference in the number of *C. africus* beetles caught among sites ($F = 7.48$; df $= 2$; $P = 0.01$). Mean separation analysis revealed no significant difference in the number of *C. africus* caught between the two sites in Limpopo Province ($P = 0.89$), whereas there was a significant difference in the number of *C. africus* caught at Enzelsberg and Last Post ($P = 0.01$) and Enzelsberg and Capricorn ($P = 0.01$). Similarly, there was a significant difference in the number of *Stenoscelis* sp. caught among all sites ($F = 5.04$; df $= 2$; $P = 0.01$). Mean separation analysis revealed no significant difference in the number of *Stenoscelis* sp. caught between the two sites in the Limpopo Province ($P = 0.94$), whereas there was a significant difference in numbers of *Stenoscelis* sp. caught between Enzelsberg and Last Post ($P = 0.01$) as well as between Enzelsberg and Capricorn ($P = 0.03$).

Seasonal flight patterns varied among the beetle species caught and among sites for certain species. *Ambrosiodmus natalensis* was caught much earlier in the year at Last Post (October to April, peaking in November; midspring to midautumn) compared to Capricorn and Enzelsberg (January to April, peaking in February; midsummer to midautumn). *Eccoptopterus spinosus* was caught during the same time of the year at Capricorn and Last Post (July to January, peaking in November; midwinter to midsummer) with *X. spinifer* caught from July to February, peaking in November, (midwinter to end of summer) at all three sites. *Premnobius cavipennis* was caught throughout the year at all three sites with peak flight from February to June (end of summer to the start of winter, peaking in March) at Capricorn, from July to September at Enzelsberg (midwinter to start of spring, peaking in September) and from July to November (midwinter to start of summer, peaking in September) at Last Post. *Xyloctonus latus* was caught from November to January (start of summer to midsummer) at all three sites.

The seasonal flight pattern of *C. africus* was very similar in Capricorn and Last Post (early summer to midautumn), with the beetles flying earlier in the year at Last Post. The flight pattern of *Stenoscelis* sp. in the North West Province was much earlier in the year (early spring to end of summer), compared to *C. africus* in the Limpopo Province (Fig. 1, only *C. africus* and *Stenoscelis* sp. shown as they are known to infest *E. ingens*). *Cyrtogenius africus* was mostly caught from November

Table 1. Mean (SE) number of beetles and total number of beetles caught (Sept 2013 to April 2015) at three sites in South Africa, and ANOVA and Student's *t*-test statistics for comparisons among sites.

Site/Total beetles	Total beetles caught	*Ambrosiodmus natalensis*	*Cyrtogenius africus**	*Eccoptopterus spinosus*
Capricorn	2597	1.75 (1.02)[a]	2.50 (1.19)[a]	46.90 (15.60)
Enzelsberg	562	1.20 (1.01)[a]	0.25 (0.55)[b]	—
Last Post	9173	10.3 (4.80)[a]	2.90 (4.52)[a]	309.8 (127.1)
Total beetles		294	113	7134
ANOVA Statistics		$F = 3.08$, df = 2, $P = 0.05$	$F = 7.48$, df = 2, $P = 0.01$	$t = 2.05$, df = 1, $P = 0.05$

*Beetle species known to infest *E. ingens*.
Same letters within a column indicate no significant difference
—Not collected

to April (peaking in March), whereas *Stenoscelis* sp. was mostly caught from September to February (peaking in December).

Temperature and relative humidity factors. There was a significant difference in the monthly mean temperatures (September 2013 to April 2015; compared among the three sites over the 20 mo period) at the Limpopo and North West sites ($F = 3.32$; df = 2; $P = 0.04$), with Capricorn being significantly different from Enzelsberg. Monthly relative humidity compared over the 20 mo, for which data were collected (September 2013 to April 2015), was significantly different between the sites in the Limpopo Province and North West Province ($F = 15.98$; df = 2; $P < 0.001$). The number of beetles caught fluctuated over the 20 mo investigated. However, only the effect of temperature was significant ($R^2 = 0.17$, $P = 0.001$). The effect of humidity was not significant ($R^2 = 0.01$, $P = 0.07$). The peak flight times for most of the beetle species coincided with the time of the year with the highest mean temperature (Figs 2, 3, 4; only *C. africus* and *Stenoscelis* shown as they infest *E. ingens*).

Discussion

Seven beetle species were trapped in stands of *E. ingens* trees in this study. Of these, only two, *C. africus* and a *Stenoscelis* sp., have previously been collected from the stems and branches of dying *E. ingens* (Van der Linde et al. 2011a, 2011c, 2016, 2017). Of the other beetles, one, *E. spinosus*, represents a new report for the African continent, whereas the remaining four are new reports for South Africa.

Cyrtogenius africus and *Stenoscelis* sp. are known to infest stressed trees (Jordal 2009, Konishi 1956). Beetles in the genus *Stenoscelis* are known to infest trees that are nearly dead or initially infested with other bark beetles, with collections recorded from Algeria, Kenya, and the Western Cape of South Africa (Konishi

Table 1. Extended.

Premnobius cavipennis	*Stenoscelis* sp.*	*Xyleborinus spinifer*	*Xyloctonus latus*
60.4 (9.00)^{ab}	0.45 (0.23)^{b}	17.9 (5.86)^{ab}	—
21.5 (4.91)^{b}	3.10 (1.24)^{a}	0.50 (0.22)^{b}	0.10 (0.10)
77.60 (25.60)^{a}	0.10 (0.07)^{b}	57.8 (20.4)^{a}	0.30 (0.21)
3187	73	1523	8
$F = 3.26$, df = 2, $P = 0.05$	$F = 5.04$, df = 2, $P = 0.01$	$F = 5.71$, df = 2, $P = 0.01$	$t = 0.87$, df = 1, $P = 0.39$

1956). *Cyrtogenius africus* was first recorded in 1988 from various *Euphorbia* spp. in Africa (Democratic Republic of Congo [formally known as Zaire], Guinea, Kenya, Tanzania, and Uganda) and again in 2009 from dead branches of *Euphorbia triangularis* Desfontaines in the Western Cape (Jordal 2009, Wood and Bright 1992). In studies of dying *E. ingens* trees, these beetles were obtained from branches of severely diseased trees (Van der Linde et al. 2011a, 2011c, 2016, 2017).

Van der Linde et al. (2017) reported different levels of *E. ingens* mortality for the sites used in this study. In that study, Enzelsberg had the highest percentage mortality (32.5%) followed by Last Post (20.9%) and Capricorn (2.5%) (Van der Linde et al. 2017). In the current study, there was no significant difference in the number of *C. africus* and *Stenoscelis* sp. (species that infest *E. ingens*), between the two sites in the Limpopo Province (Last Post and Capricorn) despite differences in *E. ingens* mortality. Furthermore, there was a significant difference in the numbers of *C. africus* and *Stenoscelis* sp. caught at Last Post and Enzelsberg with no significant difference in *E. ingens* mortality between the two sites (Van der Linde et al. 2017). A significantly greater number of *C. africus* were caught at Capricorn compared to Enzelsberg, although Capricorn had significantly lower *E. ingens* mortality.

The seasonal flight patterns and the effect of temperature and humidity on flight activity of Scolytinae and Cossoninae had not been previously investigated in relation to *E. ingens* mortality. In this study, there was a significant difference in the number of *C. africus* and *Stenoscelis* sp. captured between the two provinces that had significant differences in monthly temperature and humidity. From our results, it appears that flight activity and numbers of beetles at a site may be more related to weather and climatic conditions than levels of *E. ingens* mortality. Effects of temperature on insect flight activity is well known (Dowdy 1994, Fadamiro and Wyatt 1995, Stock et al. 2014, Tanaka et al. 1987); however, the lack of an effect by host tree mortality levels on insect activity is surprising. *Euphorbia ingens* trees rot and decompose rapidly after they have died off (due to their high moisture content),

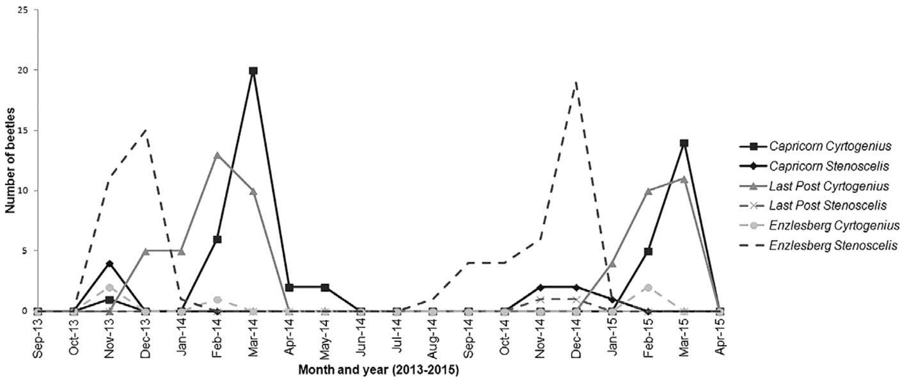

Fig. 1. Seasonal flight pattern of *Cyrtogenius africus* and *Stenoscelis* sp. caught at Capricorn, Enzelsberg, and Last Post from September 2013 to April 2015.

becoming an unsuitable host for the weevils to infest. The weevils (*C. africus* and *Stenoscelis* sp.) infest highly stressed *E. ingens* trees at the final stages of mortality (Van der Linde et al. 2016, 2017), and so the number of trees actually producing beetles at any one time may be similar among sites, because trees that have already died are not suitable for beetle production.

Although the majority of beetles trapped in this study have not been found to infest diseased or dying *E. ingens* trees, their discovery is significant because they

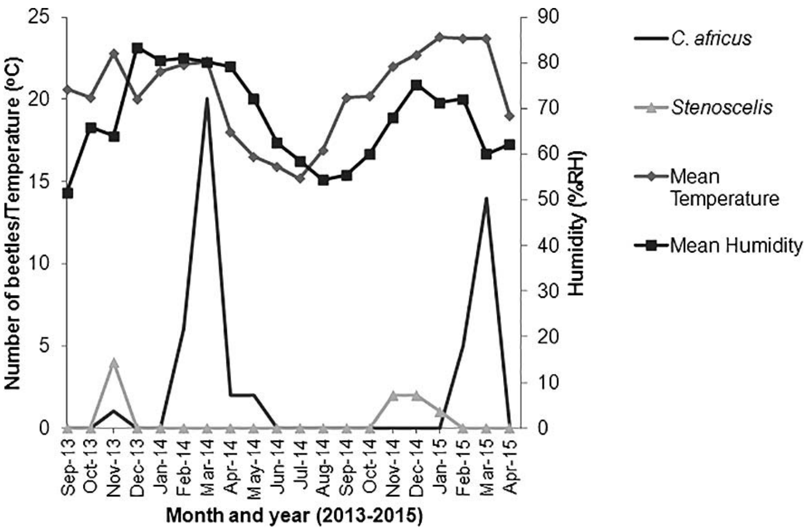

Fig. 2. Mean temperature and mean relative humidity associated with *Cyrtogenius africus* and *Stenoscelis* sp. trap captures at Capricorn from September 2013 to April 2015.

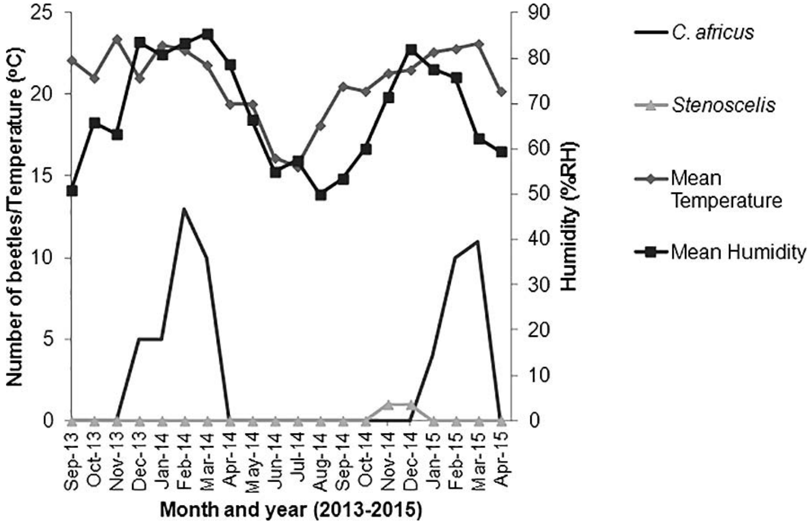

Fig. 3. Mean temperature and mean relative humidity associated with *Cyrtogenius africus* and *Stenoscelis* sp. captures at Last Post from September 2013 to April 2015.

all represent new reports for South Africa. The beetle, *E. spinosus*, is known to infest small stems and branches of coffee, cocoa, and mango, but it is not known as a primary pest; it occurs throughout the Pacific islands and most of the oriental and tropical regions (Beaver 1987, 1988, 2005, Hulcr and Cognato 2010). *Premnobius*

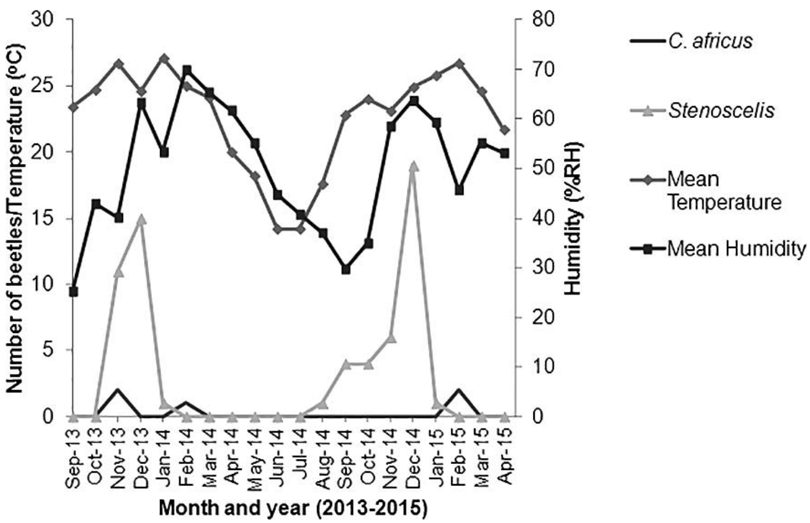

Fig. 4. Mean temperature and mean relative humidity associated with *Cyrtogenius africus* and *Stenoscelis* sp. captures at Enzelsberg from September 2013 to April 2015.

cavipennis is a polyphagous beetle native to Africa (Rabaglia et al. 2006, Wood 1977, Zanuncio et al. 2005). This beetle has been introduced into South and North America and is known to damage stressed *Eucalyptus* L'Hér trees in Brazil (Flechtmann et al. 2001, Zanuncio et al. 2005). There is very limited information available for the other beetle species collected, although all of the genera (*Ambrosiodmus* Hopkins, *Cyrtogenius* Strohmeyer, *Stenoscelis*, *Xyleborinus* Reitter, *Xyloctonus* Eichhoff) have previously been recorded in Africa (Browne 1963, Faccoli et al. 2009, Konishi 1956, Rabaglia et al. 2006, Wood 1982, Wood and Bright 1992). This study reports the first records of *A. natalensis*, *E. spinosus*, *P. cavipennis*, *X. latus*, and *X. spinifer* in South Africa. Most of these beetles are known to occur on a wide variety of hosts and they could potentially represent a threat to native and commercial trees in South Africa.

The number of *C. africus* and *Stenoscelis* sp. caught in this study was low compared to most of the other beetle species caught. Because *C. africus* and *Stenoscelis* sp. are known to infest *E. ingens*, a more specific bait for these species may need to be developed. Ambrosia beetles are commonly attracted to alcohols and volatiles released from the wood of dying and recently dead trees (Ranger et al. 2010). Stressed plants also release a variety of volatiles and some ambrosia beetles are attracted to specific host plant compounds (e.g., *Xyleborus glabratus* Eichhoff) as opposed to ethanol that is often released by decomposing plant material (Hanula and Sullivan 2008, Harrington et al. 2011). Studies of the volatiles released by stressed *E. ingens* and the response of beetles to these substances could lead to the development of more appropriate lures for these insects. This, in turn, would allow for more specific trapping and a more comprehensive knowledge of the beetles that infest and contribute to the death of *E. ingens* trees in South Africa.

Acknowledgments

We thank the Department of Science and Technology (DST), National Research Foundation (NRF) and DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB) for financial support, and Dr. Roger Beaver for assistance with beetle identification. Mr. Gert van der Merwe, Mr. Keith Johnson, and Mr. Henk Fourie are thanked for permission to use their properties for field studies.

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