METAMORPHOSIS
www.metamorphosis.org.za
LEPIDOPTERISTS' SOCIETY OF AFRICA
Volume 26: 38–43
ISSN 1018–6490 (PRINT)
ISSN 2307–5031 (ONLINE)

The ants and scale insects on which the critically endangered butterfly *Chrysoritis dicksoni* (Gabriel) (Lepidoptera: Lycaenidae: Aphnaeinae) depends for its survival

Published online: 12 September 2015

Jan H. Giliomee[1] & David A. Edge[2]

[1]Department of Botany and Zoology, University of Stellenbosch, South Africa. E-mail: jhg@sun.ac.za
[2]P. O. Box 2586, Knysna, 6570, South Africa. E-mail: orachrysops@gmail.com (corresponding author)

Abstract: The relationship between the critically endangered butterfly *Chrysoritis dicksoni* (Gabriel), the ant *Crematogaster peringueyi* Emery and scale insects (Coccoidea) is discussed. The ants collect honeydew secretions from the scale insects and feed the butterfly larvae through trophallaxis. In this study the distribution of the ant nests was studied while the scale insects involved in this tritrophic relationship were collected from satellite nests of the ants and identified for the first time. Several scale insect species were found, all from the families Coccidae (soft scales), Pseudococcidae (mealy bugs) and Keriidae (lac scales). Steps were taken towards achieving formal conservation status for one of the two remaining sites where the butterfly is found.

Key words: Coccoidea, scale insects, *Crematogaster peringueyi*, trophallaxis, honeydew.

Citation: Giliomee, J.H. & Edge, D.A. (2015). The ants and scale insects on which the critically endangered butterfly *Chrysoritis dicksoni* (Gabriel) (Lepidoptera: Lycaenidae: Aphnaeinae) depends for its survival *Metamorphosis* **26**: 38–43.

INTRODUCTION

Many species of the butterfly family Lycaenidae are associated with ants (Clark & Dickson, 1971; Pierce *et al*., 2002). In the vast majority of cases the relationship between the myrmecophilous caterpillars and the ants are mutualistic, with the caterpillars producing nutritious secretions utilised by the ants and the ants providing protection against predators and parasitoids. In some cases, however, the relationship is parasitic: the caterpillars either feed on the ant brood or they are being fed through trophallaxis by the ants, the latter being called "cuckoo-type" parasitism (Fiedler, 2012; Heath, 2014). One such cuckoo–parasite is the critically endangered butterfly *Chrysoritis dicksoni* (Gabriel, 1946).

Chrysoritis dicksoni was originally discovered near Melkboschstrand north of Cape Town where Clark & Dickson (1971) partially described its life history and established that its larvae and pupae are found in the nests of the arboreal ant *Crematogaster peringueyi* Emery. They surmised that the larvae fed on the ant larvae since in captivity they would not feed on the plants on which the eggs were laid, and ant brood was plentiful in the ant nests investigated.

Heath & Brinkman (1995) investigated the food source of the 1st and 2nd instar larvae and concluded that they were principally fed by trophallactic exchange from the attendant *C. peringueyi* ants, with a single incidence of direct predation by a larva on a scale insect nymph. Heath (1998) studied final instar larvae in captivity with ants and scale insects collected from a field colony but found no evidence of direct predation on either the scale insects or the ant brood, but observed regular trophallactic feeding of the larvae by the ants. From consistent observations of an association between the ant nests occupied by larvae and pupae and scale insects he surmised that honeydew secretions from the scale insects were an essential trophic resource for the *C. peringueyi* ant colonies, and indirectly for the *C. dicksoni* larvae.

Chrysoritis dicksoni is today only found over a small area near Witsand on the south coast of the Western Cape Province of South Africa, and it has been assessed as Critically Endangered (Mecenero *et al*., 2013). The colonies near Witsand occur on parts of the farms Westfield 483 and Westfield 478 (Fig. 1). The vegetation was described as Canca Limestone Fynbos (FFl3) by Mucina & Rutherford (2006).

Area 1 is the original place where Ernest Pringle rediscovered *C. dicksoni* near Witsand (Pringle, 1990). The owner is P.S. Uys and the farm is Westfield 478. The butterfly occurs on several "islands" of relatively intact natural vegetation where limestone pavements outcrop (Fig. 2), within grazing areas for cattle and

Received: 19 January 2015
Accepted: 12 September 2015

game (lighter colours) that have been created by removal of the limestone and the natural vegetation, and sowing of pasture crops. This area is virtually free

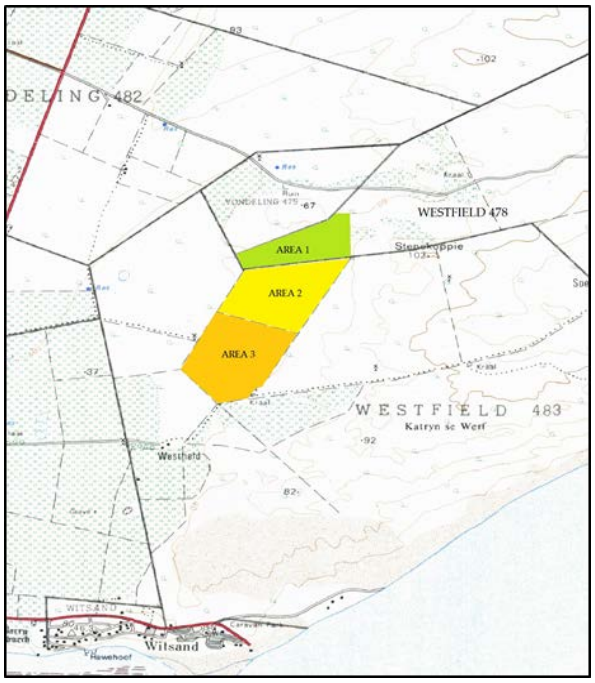

Figure 1 – The areas north of Witsand where *C. dicksoni* occurs, on the farms Westfield 478 and 483.

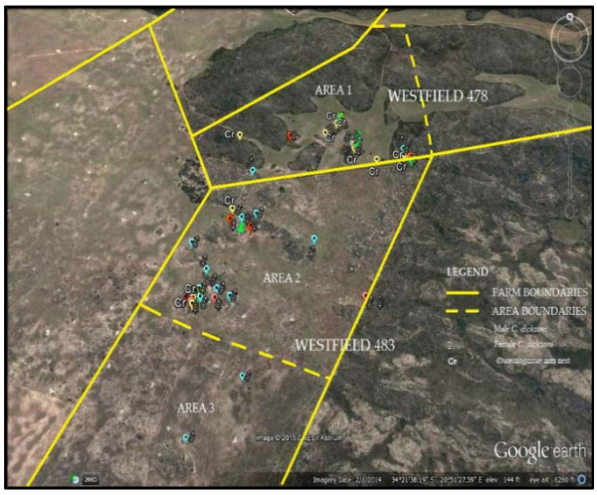

Figure 2 – Records of *C. dicksoni* on the farms Westfield 483 and Westfield 478.
Red markers = females; blue markers = males; yellow markers = *Crematogaster* ant nests

of alien plant species, thanks to the efforts of the owner, but is continuously threatened by invasion from the neighbouring Area 2, on the farm Westfield 483, which is owned by Stellenbosch University, and has several sub populations of *C. dicksoni*. The land is much more degraded than Area 1 but the butterfly persists on a number of "islands" similar to Area 1. Area 2 is heavily infested with alien plants (mainly *Acacia cyclops*) in some places and these aliens are even taking hold in the areas where the butterflies are breeding. Area 3 is also part of Westfield 483, further to the south. It is not as degraded as Area 2 (in terms of grazing or alien infestation), but there do not appear to be any breeding areas, although sporadic individuals have been encountered even as far as the large farm shed which is prominent in the middle of this area.

These sites (Areas 1, 2 and 3) have been monitored by the second author and co-workers since 2009. There was a large emergence of adults in 2009 (Curle & Ficq, 2009) across all three areas. Since that time numbers have declined, and in 2014 only three of the original six breeding sites were occupied. This period coincides with the destructive efforts to remove alien plants in Area 2, and may partially explain the reductions, but the apparent loss of two of the sub populations in Area 1 is a cause of great concern, since management practices have not been changed.

No attempt was made until 2014 to have the scale insects (Hemiptera: Coccoidea) identified by experts. They were originally referred to as "armoured scales" (Diaspididae) by Heath & Brinkman (1995), which do not secrete honeydew. It was probably a misnomer as they later refer to Coccidae, which is a family of honeydew secreting soft scales. Heath (2014) shows a picture of scale insects which "were probably wax scale of the family Coccidae", but from examination they are more likely to be scale insects of the family Keriidae.

Edge & Terblanche (2010) described a research programme with the aim of filling the gaps in the known autecology of *C. dicksoni*, describing its synecology and developing a conservation management plan. They were subsequently appointed as custodians of *C. dicksoni* under the COREL programme (Edge, 2011). As part of this ongoing study, funded by the Brenton Blue Trust, the authors have investigated the distribution of *C. peringueyi* nests in the area where *C. dicksoni* is found; the plants within which these nests occur; and have recorded and identified scale insects from which the ants are collecting honeydew.

MATERIALS AND METHODS

Between 2009 and 2014 plants on the two sites (Areas 1 and 2 near Witsand – Fig. 1) where adults of *C. dicksoni* occur were thoroughly searched for nests of *C. peringueyi*, which potentially harboured larvae of *C. dicksoni*, and details of each nest were recorded (see Table 1). During September 2014, when nests were found, the rest of the plant and surrounding plants were searched for the small satellite nests which are often present near the main nest and harbour scale insect colonies. A selection of these satellite nests (about one in three) were collected by cutting off the branch with pruning shears and placed in plastic bags. In the laboratory, these were placed in the ice cubicle of a domestic refrigerator for c. 15 minutes to immobilise the ants so that the nests could be opened to collect the scale insects inside.

RESULTS

A record of all the main active *C. peringueyi* ant nests occurring in the localities where *C. dicksoni* adults fly

was made by the second author over several years and appears in Table 1. The larger carton nests were up to 40x25x20 cm in size and were constructed from a combination of leaves, grass and twigs. Two thirds were built at ground level, and may have extended underground, with the other one third entirely aerial, supported in the branches of the host plants (Fig. 3). The most popular host plant was the shrub *Searsia glauca* (Thunb.) Moffett (11 records), followed by the restios *Thamnocortus pluristachyus* Mast (3) and *Restio leptocladus* Mast. (2).

D.A. Edge

Figure 3 – Typical carton nest made by *Crematogaster peringueyi*, the host ants of *Chrysoritis dicksoni*, in a *Searsia crenata* shrub c. 80 cm from ground level.

The second author was investigating ant nest no. 3/1 on 24th August 2010, built at ground level between the stalks of a "cut" restio *T. pluristachyus*, under the shade of a *S. glauca* shrub. Many (c. 12♂ and 1♀) *C. dicksoni* adults were flying within 10 m of the nest. On top of the nest orange colour was seen, which inspection revealed to be wing fragments. Next to this was a freshly eclosed ♂ *C. dicksoni* whose wings had not yet expanded, with its underside camouflaging it very well. It was crawling towards a ♀ whose wings were almost fully expanded. The ♂ moved alongside and copulation commenced, continuing for c. 30 minutes (Fig. 4).

R.F. Terblanche

Figure 4 – Freshly eclosed pair of *C. dicksoni* copulating on top of *C. peringueyi* ant nest. Wing fragments visible above mating pair.

During this time a second ♀ emerged and rested on the nest, expanding its wings. Many ants were present but did not molest the butterflies. An ant was then seen carrying a wing fragment in its jaws and placing it in the pile. These fragments may well be wings of butterflies that had failed to fully eclose in the nest.

The first author collected thirteen satellite ant nests in early September 2014, eight in Area 1 and five in Area 2 (Fig. 1). One of the satellite nests in Area 1 was devoid of scale insects and does not appear in Table 2; but the others all contained one or more individuals (Table 2). No butterfly larvae or pupae were present in any of the satellite nests. Three of these nests (S2–4) were hosted by *S. glauca*, and harboured only post-oviposition adults of soft scales (Coccidae) which could not be identified to specific level with any certainty. Those in nest S3 were probably *Coccus hesperidum* L. The satellite nests S1 and S5 were hosted by *Metalasia dregeana* D.C. by contrast, and supported thriving scale insect populations with adults and nymphs of the lac scale *Tachardina minor* (Brain) and an undescribed species of the mealybug genus *Paracoccus* (Pseudococcidae), the latter with about 80 individuals.

In Area 1, four out of seven of the satellite nests were found on *Muraltia spinosa* (L.) F. Forest & J.C. Manning, a thorny shrub which is hardly seen in Area 2. Nest W1 had about 60 specimens of mealybugs and another (W6) had 16 individual mealybugs (Pseudococcidae) of a similar species, also an undescribed *Paracoccus* species, occurring together with about 140 soft scale insects (Coccidae species). The latter were mostly old adults with eggs or crawlers, but some were still pre-oviposition. Similarly, the c. 100 soft scale insects in nest W3 had eggs or crawlers underneath their bodies. Nest W2 had c. 10 soft scale insects of various ages. Nest W4 had a single specimen of a young adult white wax scale, a *Ceroplaste*s sp. of the *C. rusci* group (C. Hodgson, pers. comm.), and in nest W5 one adult soft scale insect with eggs and 19 younger individuals were found. All the soft scale insects of nests W2, W3, W5 and W6 belonged to the genus *Saissetia* (Coccidae).

All three families of the Coccoidea found in these nests secrete honeydew, which is a carbohydrate-rich source of food for the ants, which in turn afford the scale insects protection in a mutualistic symbiotic relationship. Presumably this also serves (indirectly) as a source of food for the larvae of *C. dicksoni*, which live in the larger nests containing their own populations of Coccoidea (Giliomee pers. obs.). None of these larger ant nests were collected, on account of the Critically Endangered status and rarity of *C. dicksoni*.

The Coccoidea found in ant nests at area 1 were usually more numerous than at area 2, which correlates with the larger number of *C. dicksoni* observed in Area 1.

DISCUSSION

Because of the authors' reluctance to break open ant nests it remains uncertain as to which ant nests are preferred by the *C. dicksoni* early stages, and whether for example "aerial" nests are preferred over nests built on the ground. The only definite evidence was the nest from which the *C. dicksoni* adults were emerging and this was indeed built on the ground, as in fact were two thirds of the ant nests recorded.

Heath (pers. comm.) has reported that all the ant nests demonstrably associated with *C. dicksoni* at the Pella site near Mamre in the late 1980's and early 1990's (also in 1998 at the Witsand area he visited) were "stalagmite-like" and built on the ground – never above ground in a shrub as ant nests are often found elsewhere. He didn't recall any scale insects being seen in either of the two "stalagmite" ant nests they opened at Pella or in the one "stalagmite" ant nest opened at a Witsand locality. There were however ant trails in the early morning of ants to collecting secretions from the scale insects several meters away. He also said that at Pella the "stalagmite-type" ant nests vanished at the same time that *C.dicksoni* did, and suggests this was not a coincidence.

The results of this survey give a good indication of the scale insects in the nests of *C. peringueyi* on which the larvae of *C. dicksoni* (indirectly) depend for the food they receive from the ants through trophallaxis. Nearly all the small "satellite" nests contained scale insects, sometimes in large numbers, and this would presumably also be the case for the large "mother" nests. The latter could not be sampled as their destruction would place the food source of the larvae and therefore the survival of the butterfly in jeopardy. Since no other food source for the butterfly larvae is known, their survival appears to be dependent on the presence of ant nests containing honey dew secreting scale insects.

It is not clear why the distribution of *C. dicksoni* is restricted to the two small localities near Witsand (and previously the area near Mamre where it was found up until the mid 1990s). A survey by Giliomee (2014) showed that nests of *C. peringueyi* containing scale insects occur over a wide area along the western and southern coasts of South Africa. In this respect *C. dicksoni* is similar to several other endangered butterfly species in South Africa, such as *Orachrysops niobe* (Trimen) and *Thestor brachycerus brachycerus* (Trimen), where the distribution is confined to very small areas (Mecenero *et al*. 2013) and the species do not expand their territories.

The host plants used by the ants and the honeydew secreting scale insects are threatened by the presence of the invasive alien *Acacia cyclops* A.Cunn. ex G.Don. (Rooikrans) at the site. During this study the importance of establishing formal conservation status for at least one of the two sites on which the species is known to occur was again realised. A meeting was arranged between representatives of the landowner (Stellenbosch University), the lessee, the Lepidopterists' Society of Africa and CapeNature, to find the best way to conserve the site and to implement a management plan (see below). These steps are consistent with the comments by Terblanche & Van Hamburg (2003) who stated that this butterfly is "in the process of vanishing if conservation strategies are not implemented in the near future".

MANAGEMENT RECOMMENDATIONS

Alien plants

Acacia cyclops (Rooikrans) is ubiquitous in Area 2. In some places they form dense stands, which the tenant farmer has attempted to control unsuccessfully by mechanical means (using a tractor to dig out the tap roots of the Rooikrans). This has not only caused a lot of collateral damage to other plants, including potential and actual ant nest sites, but also despite this effort there are now many juvenile Rooikrans growing again. These can only be controlled and eventually eradicated manually, for which the tenant farmer does not have the resources in terms of trained and supervised labour. The most urgent need was for the juvenile Rooikrans springing up at the prime butterfly breeding site (c. 1.5 ha) to be removed by hand. Fortunately CapeNature agreed to assist; the second author marked out this area; and the work was completed by CapeNature staff with contract labour in July 2015. Further areas will be cleared during late 2015 and 2016. Biological control agents could be introduced (which have no harmful effect on the butterflies or their symbionts) by CapeNature as part of the eventual solution of the problem.

Grazing animals

Grazing of cattle, sheep and even ostriches has been practised across Areas 2 and 3 in the past. It is unclear what impact these grazing practices have had on the butterfly, but it is recommended that all grazing ceases (as a precautionary measure) for a few years so it can be seen if the butterfly populations recover. Areas 2 and 3 are surrounded by a good livestock fence so exclusion should be simple to achieve.

ACKNOWLEDGMENTS

The authors are grateful to the landowners, Stellenbosch University and P.S. Uys, for permission to conduct this research on their properties. I. Millar of the South African National Collection of Insects, Pretoria, and C. Hodgson of the National Museum of Wales, Cardiff kindly examined the scale insect samples and determined names wherever possible. CapeNature personnel R. Hiseman and M. Hanson are thanked for their diligence in assisting with alien clearance. A. Heath also kindly shared some of his observations on *C. dicksoni*, which has shed more light on its ecology. R.F. Terblanche assisted with field work in the early part of this project, providing much inspiration and ideas, and would have been a co-author were it not for his other commitments.

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Table 1: *Crematogaster peringueyi* Emery ant nests found adjacent to where *Chrysoritis dicksoni* adults were flying

Nest no.[1]	Coordinates		Dates	Ant nest size[2]	Base height[2]	Ant nest made from	Ants[3]	Brood[3]	Host plants	
	S	E							Species[4]	Height[2]
1/1	34°21'22.8"	20°51′37.9″	2010.08.24	40x25x15	0	Leaves, grass	Yes	Yes	Segl	200
2/1	34°21'20.3"	20°51′35.7″	2010.08.05	20x10x10	0	Leaves, twigs	Yes	No	-	-
2/2	34°21′20.6"	20°51′35.7″	2010.08.05	20x10x10	0	Leaves	No	No	Dead shrub	60
2/3	34°21′21.0"	20°51′34.8″	2010.08.05	35x25x20	0	Leaves	Yes	Yes	Rele, Mupa	130
2/4	34°21′21.0"	20°51′34.6″	2010.08.05	40x25x20	30	Grass, leaves	Yes	No	Segl	110
2/5	34°21′20.6"	20°51′34.7″	2010.08.24	25x15x15	0	Leaves	Yes	Yes	Thpl, Paga	100
2/6	34°21′19.9"	20°51′35.4″	2011.08.17	15x10x10	0	Leaves	Yes	No	Segl	120
3/1	34°21′24.3"	20°51′37.5″	2010.08.24	30x25x15	0	Leaves, restio	Yes	Yes	Segl, Thpl	200
3/2	34°21′24.3"	20°51′37.5″	2010.08.24	35x15x15	15	Leaves	Yes	No	Segl	160
3/3	34°21′23.6"	20°51′38.1″	2011.08.17	20x15x15	0	Leaves, grass	Yes	No	Segl	190
3/4	34°21′25.2"	20°51′37.0″	2011.08.17	15x20x15	0	Leaves	Yes	No	Rele	120
3/5	34°21′24.9"	20°51′37.7″	2012.08.28	30x30x25	20	Leaves, twigs	Yes	Yes	Segl, Pttr	200
3/6	34°21′24.9"	20°51′37.7″	2012.08.28	40x15x25	0	Grass, leaves	Yes	No	Thpl	120
3/7	34°21′23.8"	20°51′37.7″	2012.08.29	25x20x15	0	Leaves	Yes	No	Segl	130
4/1	34°21′26.0"	20°51′47.4″	2010.08.05	10x15x10	10	Grass	Yes	No	Paga	80
4/2	34°21′26.3"	20°51′47.4″	2010.08.05	20x15x15	0	Leaves	Yes	No	Hepe	50
4/3	34°21′26.2"	20°51′46.6″	2010.08.05	35x30x25	0	Leaves	Yes	Yes	Segl	80
4/4	34°21′26.1"	20°51′45.6″	2011.08.17	25x20x15	10	Grass, twigs	Yes	No	Segl	120
6/1	34°21′45.9"	20°51′10.3″	2014.09.04	30x25x20	80	Grass, leaves	Yes	No	Segl	150
6/2	34°21′45.3"	20°51′11.1″	2014.09.04	20x15x15	0	Leaves, grass	Yes	No	Hete	50
6/3	34°21′45.9"	20°51′10.3″	2014.09.04	40x25x20	80	Leaves, twigs	Yes	Yes	Secr	220

1 Nest nos. = Relevé/no. See Fig. 1 for location of relevés.
2 All dimensions are in cm; size of nests given as height x length x width
3 Ants in all cases *Crematogaster peringueyi* Emery
4 Plant name abbreviations: Segl = *Searsia glauca* (Thunb.) Moffett; Rele = *Restio leptocladus* Mast.; Mupa = *Muraltia pappeana* Harv.; Thpl = *Thamnocortus pluristachyus* Mast.; Paga = *Passerina galpinii* C.H. Wright; Pttr = *Pterocelastrus tricuspidatus* (Lam.) Sond.; Hepe = *Helichrysum* cf *petiolare* Hilliard & B.L. Burtt; Hete = *Helichrysum teretifolium* (L.) D.Don; Secr = *Searsia crenata* (Thunb.) Moffett

Table 2. Coccoidea collected in satellite nests of *Crematogaster peringuyei* Emery on various host plants at the farms Sandfontein = Westfield 478, Area 2 (S) and Westfield 481, Area 1 (W)

Sites	Coordinates		Dates	Sat. nest size[1, 2]	Base height[1]	Ant nest made from	Scale Insects (Coccoidea)		Host plants	
	S	E					Fam.[3]	Species[4], comments	Species[5]	Height[1]
S1	34°21′27.0"	20°51′41.0″	2014.09.02	12x10x5	20	Leaves	Ke	Tami, various ages	Medr	70
S2	34°21′27.0"	20°51′40.4″	2014.09.02	10x8x6	10	Grass, twigs	Co	Reddish, 2♀, 1♂, old adults	Segl	130
S3	34°21′21.0"	20°51′34.6″	2014.09.02	8x8x6	30	Grass, leaves	Co	Sasp., old adults	Segl	110
S4	34°21′26.9"	20°51′41.6″	2014.09.03	10x12x8	50	Grass & twigs	Co	Cohe, one old adult	Segl	90
S5	34°21′22.8"	20°51′32.9″	2014.09.03	15x10x10	0	Leaves, grass	Ps	Many adults and nymphs	Medr	60
W1	34°21′45.9"	20°51′10.3″	2014.09.04	12x12x10	50	Leaves, grass	Ps	Many adults and nymphs	Musp	90
W2	34°21′45.3"	20°51′11.1″	2014.09.04	10x10x8	0	Grass	Co	Black, young adults/nymphs	Muca	30
W3	34°21′45.6"	20°51′11.7″	2014.09.04	10x10x5	20	Grass, twigs	Co	Mostly adults with eggs	Asbu	40
W4	34°21′45.9"	20°51′10.3″	2014.09.04	15x12x10	70	Grass, leaves	Co	Ceru, 1 young adult	Secr	220
W5	34°21′45.4"	20°51′10.5″	2014.09.04	12x10x8	0	Leaves	Co	Mostly nymphs	Musp	15
W6	34°21′45.6"	20°51′11.0″	2014.09.04	10x8x8	10	Grass, leaves	Co	Mostly adults with eggs	Musp	20
W7	34°21′45.8"	20°51′10.9″	2014.09.04	12x12x8	10	Grass	Ps	Adults and nymphs	Musp	25

1 All dimensions are in cm; size of satellite nests given as height x length x width
2 Ants in all cases *Crematogaster peringueyi* Emery
3 Family abbreviations: Ke = Keriidae (Lac scale); Co = Coccidae (Soft scale); Ps = Pseudococcidae (Mealy bugs)
4 Scale insect abbreviations: Tami = *Tachardina minor*; Sasp. = *Saisettia* sp.; Cohe = *Coccus hesperidum*?; Ceru = *Ceroplastes* sp. of *C. rusci*. L. group. Closest to *C. eugeniae* but does not quite fit that either. (C. Hodgson, pers. comm.)
5 Plant name abbreviations Medr = *Metalasia dregeana* D.C.; Segl = *Searsia glauca* (Thunb.) Moffett; Musp = *Muraltia spinosa* (L.) F.Forest & J.C.Manning; Muca = *Muraltia calcicola* Karis; Asbu = *Asparagus burchellii* Baker; Secr = *Searsia crenata* (Thunb.) Moffett