Morphological and reproductive consequences of an anther smut fungus on *Oxalis*

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Thecaphora capensis, an anther smut fungus from the Greater Cape Floristic Region (GCFR) of South Africa infects a number of *Oxalis*, the largest geophytic genus in this area. Diseased individuals produce fungal spores instead of pollen in their anthers, which allows for pollinator-mediated transmission of spores. We build on existing meagre knowledge of this plant-pathogen system by elucidating the known host range of *T. capensis* and by assessing its effect on host morphology and reproduction. Three new hosts were identified, bringing the total number of known host species to twelve. Infection of *O. incarnata*, *O. lanata* and *O. nidulans* generally has negative effects on all morphological traits assessed. However, the magnitude of effect on various characters varied between populations and hosts. Fungal spore presence on stigmatic surfaces of healthy *O. incarnata* and *O. lanata* did not compromise seed set. However, diseased individuals were usually sterile, indicating that *T. capensis* has major population-level impacts. Determining the full host range and consequences of infection are essential, as it will allow for comparisons with similar systems to formulate and test general hypotheses of vector-borne disease dynamics.

Keywords: spore-transmission, *Microbotryum violaceum* s. l., Greater Cape Floristic Region, *Thecaphora capensis*.

The Greater Cape Floristic Region (GCFR) (Born *et al.* 2006) at the southwestern tip of Africa is acknowledged as one of the world's richest floristic regions (Goldblatt & Manning 2000, Myers *et al.* 2000, Linder 2003). It is particularly rich in geophytes (Born *et al.* 2006), with the genus *Oxalis* L. (Oxalidaceae) alone containing ca. 200 species (Oberlander *et al.* 2009). Leaves and flowers of *Oxalis* emerge only during the wet winter months in the region (April–August) (Dreyer *et al.* 2006) and die back to the bulbs in spring; the plants remain dormant underground for the remainder of the year.

Salter (1938) was the first to report a flower-associated smut fungus from *Oxalis lanata* var. *rosea* T. M. Salter ("…it is often affected by a species of smut which attacks the anthers and bulbs …"). This fungus was re-collected 70 years later and described as distinct species *Thecaphora capensis* Ro-

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ets & L. L. Dreyer (Roets *et al.* 2008). *Thecaphora capensis* (Basidiomycota, Glomosporiaceae) forms masses of dark spores that replace pollen within the anthers of infected plants (Roets *et al.* 2008). Production of spores in this specialised niche implicates flower visitors as dispersal agents (Curran *et al.* 2009). Interestingly, some *Oxalis* species from Europe, Asia and America host the currently recognised sister species of *T. capensis*, *T. oxalidis* (Ellis & Tracy) M. Lutz, R. Bauer & Piątek (Roets *et al.* 2008, Vánky *et al.* 2008). This species forms teliospores in the seed capsules of its hosts rather than in the anthers (Ellis & Tracy 1890, Vánky 2012).

Flowers are considered an excellent habitat for many fungi (Brysch-Herzberg 2004), since they contain abundant nutrient resources intended for host reproduction (Ngugi & Scherm 2006). Pathogens have the ability to directly affect host fecundity and viability when they exploit these resources (Dobson & Crawley 1994). Curran *et al.* (2009) showed that *T. capensis* had negative morphological effects on *O. incarnata* L. Although sample sizes were limited, results showed that infected plants displayed smaller petal and leaf surface areas and shorter styles and stamens compared to flowers of healthy plants. Infected *O. incarnata* bulbs that were surface sterilised still produced spores in the anthers in the following flowering season, suggesting that the infection is systemic and that the fungus probably resides in the bulbs during plant dormancy. Additionally, infected *O. lanata* Thunb. plants displayed an almost complete failure to reproduce sexually.

Species in the Caryophyllaceae also often host an anther smuts referred to as *Microbotryum violaceum* (Pers.) G. Deml. & Oberw. (Elmqvist *et al.* 1993, Shykoff *et al.* 1997, Biere & Honders 1998, Bucheli & Shykoff 1999, López-Villavicencio *et al.* 2007), which is a species complex that recently was split into a number of host specific and predominantly cryptic species (Chlebicki & Suková 2005; Lutz *et al.* 2005, 2008; Denchev 2007 a, b; Denchev *et al.* 2009; Denchev & Denchev 2011; Piątek *et al.* 2012). Diseased plants usually display morphological differences between healthy and diseased flowers (Baker 1947) congruent with those observed in *O. incarnata* by Curran *et al.* (2009). Similar to *O. lanata*, plants in the Caryophyllaceae infected with *M. violaceum* s. l. lose their ability to reproduce and their flowers solely function as site for fungal reproduction (Marr 1997). *Microbotryum violaceum* has also been shown to stimulate the production of additional flowers in some infected hosts (Lee 1981, Jennersten 1988, Alexander & Maltby 1990). The fungus may thus alter the reproductive characteristics of its host to promote its own transmission. In addition, the mere presence of spores on stigmas of healthy flowers may negatively impact host reproduction even when this does not necessarily lead to infection (Alexander 1987, Randall & Hilu 1990, Marr 1997, Marr 1998). These disease characteristics may have enormous consequences on the evolutionary biology of the host plants.

Very little is currently known about the effect of *T. capensis* on *Oxalis* (Curran *et al.* 2009), but it may share numerous characteristics with the *Microbotryum*-Caryophyllaceae system. In both systems the disease functions as a sexually transmitted disease with spore transmission between flowers

mainly via pollinators. Like the best studied hosts of *Microbotryum*, *Silene latifolia* Poir. and *S. dioica* (L.) Clairv., GCFR *Oxalis* species are perennial and may live longer than 40 years (Salter 1944). These two systems may thus prove interesting for the study of comparative biological, ecological and evolutionary influences posed by flower-infecting fungi on plants in general.

The study by Curran *et al.* (2009) initiated investigations into the ecology and host range of *T. capensis* on GCFR *Oxalis*. However, due to limited sample sizes (assessing only a few individuals from a single population of a single species), general morphological and reproductive effects of the fungus on *Oxalis* could not be drawn. The present study aims to address this shortcoming by assessing morphological differences between healthy and diseased individuals in three test species and from numerous populations. We assessed the intra-population reproductive effects of *T. capensis* infection on *O. incarnata* and *O. lanata,* and determine whether the presence of fungal spores on healthy flowers affects seed set. Finally, we build on an existing data base of infected *Oxalis* populations within the GCFR to elucidate the known host range and extent of occurrence of *T. capensis*.

Materials and methods

Morphological effects of infection on host

Healthy and diseased individuals from infected *O. incarnata*, *O. lanata* and *O. nidulans* Eckl. & Zeyh. populations were compared to test the morphological effect of *T. capensis* on its host. Four infected populations were found for *O. incarnata*, five for *O. lanata* and one for *O. nidulans* (Tab. 1). Whole plants (all above-ground plant parts) (n = 20 healthy and 20 diseased individuals, where available) were collected from these populations for morphological comparisons. For each of these plants the total number of flowers per plant and total dry mass was recorded. Additional flowers and leaves (from 20 healthy and 20 diseased individuals, where available) were also collected from each of these populations and the following measurements were taken: style length, stamen length and petal and leaf surface areas. All southern African *Oxalis* displays a tristylous breeding system, in which individuals in populations produce flowers conforming to one of three different floral morphs (Long, Mid or Short, depending on the position of the stigma) (Salter 1944). In order to standardise our measurements, we only considered Long morph individuals. Lengths of styles and longest stamens were measured using electronic callipers. Single petal and leaf circumferences were traced onto transparency film and later filled with an overhead projection marker. These pictures were cut out and their surface areas measured using a planimeter (Model LI-3000, Lambda Instrument Corporation, USA).

Data were analysed for each population separately and thereafter combined in a single analysis for each host. All morphological data were analysed in Statistica 10 (StatSoft Inc, Tulsa, OK, USA) using t-tests for normally distributed data, and Mann-Whitney U-tests for non-parametric data.

We corrected for alpha-inflation in large repeated-test tables using step-up False Discovery Rate (FDR) adjustments (Benjamini & Hochberg 1995, Garcia 2004).

Tab. 1. Localities of all known *Thecaphora capensis*-infected *Oxalis* species and populations. na = not applicable.

Species	Location	Degrees South	Degrees East
Oxalis bifida	Cultivated specimen	na	na
Oxalis ciliaris	Bonnievale	33° 56.520	20° 02.733
Oxalis depressa	Bonnievale	33° 59.533	20° 11.299
Oxalis eckloniana	Ceres, Gydo Pass	33° 14.371	19° 19.935
Oxalis engleriana	Jonaskop	33° 57.096	19° 31.056
Oxalis glabra	Caledon	34° 05.342	19° 33.077
Oxalis incarnata	Knysna Phantom Pass	34° 00.623	23° 00.199
"	Knysna Homtini Pass	33° 57.144	22° 54.840
"	Knysna Gouna	33° 59.590	23° 02.270
"	Cecilia Forest	33° 59.812	18° 25.339
Oxalis lanata	Stellenbosch Mountain	33° 56.657	18° 52.815
"	Jonkershoek 1	33° 59.400	18° 57.873
"	Paradyskloof	33° 58.039	18° 51.954
"	Brandwacht	33° 57.918	18° 52.624
"	Constantia	34° 00.235	18° 24.532
Oxalis nidulans	Betty's Bay	34° 19. 384	18° 57.838
Oxalis polyphylla	Hermanus	34° 23.999	19° 15.425
Oxalis tenella	Clanwilliam	32° 21.057	18° 55.910
Oxalis truncatula	Jonkershoek	33° 59.539	18° 58.730

Reproductive effects of infection on host

The reproductive potential of healthy and diseased individuals was compared in the 9 populations of *O. incarnata* and *O. lanata* mentioned above. For each diseased population, 20 healthy and 20 diseased plants (where available) were hand pollinated in the field. Only legitimate crosses were made using pollen from young, newly-opened flowers between healthy plants. Before pollen transfer, flowers were examined for the presence of fungal spores on their stigmas using a hand-held magnifying glass. Flowers with spores on their stigmas were discarded. Hand-pollinated flowers were covered with fine gauze to control for external flower visitors. Seeds produced by flowers covered with fine gauze were collected three to four weeks later. The number of seeds produced per capsule by healthy and diseased plants was counted and compared using a multiple comparisons of mean ranks test of the Kruskal-Wallis ANOVA procedure in Statistica 10 (StatSoft Incorporated, Tulsa, USA).

For each of the infected populations of *O. incarnata* and *O. lanata*, a further 20 healthy plants were selected to investigate the effect of the presence of fungal spores on healthy stigmatic surfaces on seed set. Both fungal spores and legitimate pollen from sympatric individuals were applied to the stigmas of one flower per plant. These flowers were again covered with fine

gauze to exclude other pollinators. Seed collecting and counts per capsule proceeded as mentioned above. Counts from this experiment were compared to the number of seeds produced per capsule following hand pollination using the mean ranks test of Kruskal-Wallis ANOVA as previously described.

Results

A further three host species of *T. capensis* were identified during this study, namely *O. nidulans, O. polyphylla* Jacq. and *O. truncatula* Jacq. This brings the total number of known host species to 12, with *O. bifida* Thunb., *O. ciliaris* Jacq., *O. depressa* Eckl. & Zeyh., *O. eckloniana* C. Presl, *O. engleriana* Schltr., *O. glabra* Thunb., *O. incarnata, O. lanata* and *O. tenella* Jacq. previously identified as hosts (Curran *et al.* 2009; Tab. 1). Diseased *Oxalis* populations and species were found widespread throughout the GCFR (Fig. 1). Anthropogenic disturbance was evident at nearly all infected populations and included signs of recent fires, the clearing of roadside cuttings or the cutting of grass. Interestingly, most infected populations displayed a noticeable degree of shade cover with infected individuals more often found clustered under larger trees or shrubs than healthy individuals in the same populations.

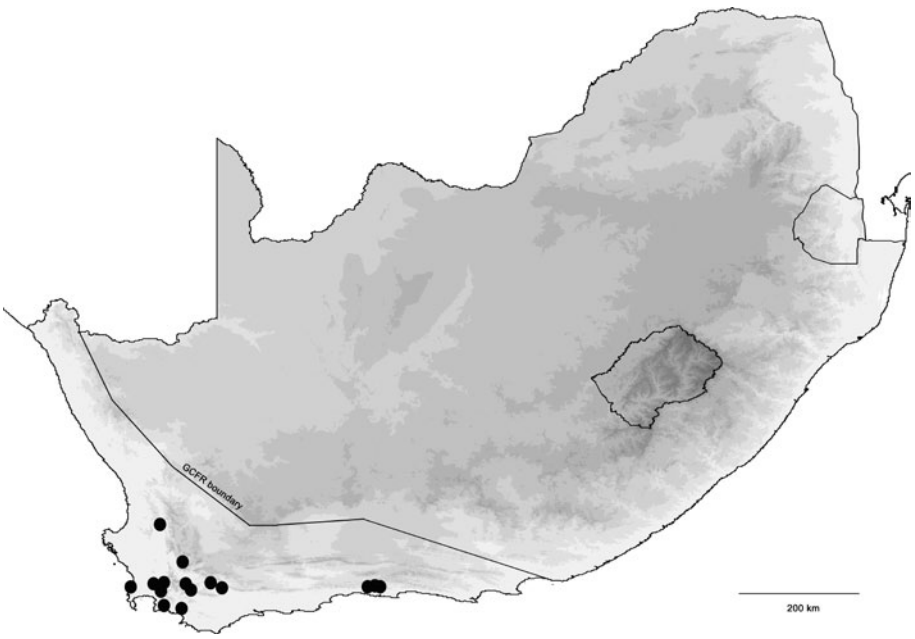

Fig. 1. Localities of all known populations of *Oxalis* infected by *Thecaphora capensis* in the Greater Cape Floristic Region (GCFR) of South Africa.

Morphological effects of infection on host

Infected *O. lanata* populations showed many floral mutations. Although not explicitly tested, it appears as though flowers may display varying degrees of disease expression, with some flowers displaying extreme mutations probably as a result of the disease. These mutations included styles within otherwise visually healthy flowers that radiated outwards and sepal-like structures growing as additional whorls within the petals of infected flowers. A few flowers contained anthers with seemingly healthy pollen in combination with others containing fungal spores.

Thecaphora capensis had drastic negative effects on *O. incarnata*, *O. lanata* and *O. nidulans* plants. Diseased plants in general produced significantly fewer flowers compared to healthy plants (Tab. 2); in the few cases when the opposite pattern was observed, the effect was never significant. Diseased plants usually had a significantly lower total dry mass and shorter style and stamen lengths (Tab. 2). Petal and leaf surface areas were usually also significantly smaller in diseased plants. The effect of disease on all measured morphological variables varied between different populations; however style and stamen dwarfing was constantly observed in all species and populations. Diseased *O. lanata* individuals also produced consistently smaller petal surface areas across all populations (Tab. 2).

Reproductive effects of infection on host

Overall, legitimate crosses using pollen from healthy plants produced significantly more seeds in healthy flowers of *O. lanata* and *O. incarnata* compared to diseased flowers (Tab. 3). Diseased *O. incarnata* plants did not produce seeds, whilst diseased *O. lanata* plants produced single seeds on only two occasions (Tab. 3). Natural seed set was also usually significantly higher than seed set in hand-pollinated diseased plants. Seed set in healthy hand-pollinated flowers and naturally pollinated flowers did not differ significantly for *O. incarnata*. However, hand-pollinated *O. lanata* flowers on average produced significantly more seeds than naturally pollinated flowers. Overall, the number of seeds produced by healthy flowers inoculated with a spore/pollen mixture did not differ significantly from flowers that received legitimate pollen only. This was true for all populations of *O. incarnata* and all except one population (Brandwacht) of *O. lanata* (Tab. 3).

Discussion

The known host range of *T. capensis* has been expanded by three species. This suggests that more hosts may await discovery, and emphasizes the need for continuous monitoring of *Oxalis* populations in the GCFR. The known hosts are scattered across the phylogeny of southern African *Oxalis* (Oberlander *et al.* 2011; data not shown). Given the broad geographic and phylogenetic spread, it is possible that most *Oxalis* species may be susceptible to infection by *T. capensis*.

Tab. 2. Morphological effect of *Thecaphora capensis* infection on *Oxalis incarnata* and *Oxalis nidulans* in various populations. Results reported as either mean ± standard deviation (parametric data) or median ± standard deviation (nonparametric data). Significance is reported (*) when the adjusted P ≤ 0.05. ns = not significant. df = degrees of freedom.

Character	Healthy	Diseased	Test value (df)	P
Oxalis incarnata				
Cecilia Forest				
Number of flowers	7.65 ± 5.25	5.70 ± 3.55	U (38) = 161.50; Z = 1.02	ns
Total dry mass (g)	0.61 ± 0.67	0.28 ± 0.17	U (38) = 118.00; Z = 2.20	*
Style length (mm)	8.02 ± 0.37	6.86 ± 0.37	U (38) = 10.00; Z = 5.12	*
Mid level stamen (mm)	4.95± 0.35	3.25 ± 0.29	t (38) = 16.32	*
Petal surface area (cm²)	0.59 ± 0.13	0.41 ± 0.10	t (38) = 4.64	*
Leaf surface area (cm²)	2.81 ± 1.09	1.79 ± 0.58	U (38) = 105.50; Z = 2.54	*
Homtini Pass				
Number of flowers	5.65 ± 3.23	3.30 ± 1.78	t (38) = 2.84	*
Total dry mass (g)	0.09 ± 0.04	0.08 ± 0.04	U (38) = 156.00; Z = 1.17	ns
Style length (mm)	7.23 ± 0.27	4.51 ± 1.53	U (38) = 4.00; Z = 5.28	*
Mid level stamen (mm)	4.86 ± 0.36	3.02 ± 0.32	t (38) = 16.78	*
Petal surface area (cm²)	0.50 ± 0.14	0.49 ± 0.12	U (38) = 186.5; Z = 0.35	ns
Leaf surface area (cm²)	1.33 ± 0.37	1.13 ± 0.45	U (38) = 121.00; Z = 2.12	*
Gouna				
Number of flowers	5.10 ± 3.74	4.00 ± 2.33	U (38) = 173.50; Z = 0.70	ns
Total dry mass (g)	0.13 ± 0.07	0.11 ± 0.09	U (32) = 118.50; Z = 0.88	ns
Style length (mm)	7.11 ± 0.69	4.92 ± 1.41	U (38) = 46.50; Z = 4.13	*
Mid level stamen (mm)	4.83 ± 0.34	3.22 ± 0.32	t (38) = 15.09	*
Petal surface area (cm²)	0.43 ± 0.13	0.39 ± 0.09	U (38) = 171.5; Z = 0.75	ns
Leaf surface area (cm²)	1.51 ± 0.44	1.51 ± 0.62	U (38) = 131.00; Z = 1.85	ns
Phantom Pass				
Number of flowers	4.65 ± 3.81	3.25 ± 2.61	U (38) = 154.00; Z = 1.23	ns
Total dry mass (g)	0.24 ± 0.17	0.16 ± 0.11	U (38) = 145.00; Z = 1.47	ns
Style length (mm)	7.54 ± 0.39	4.91 ± 1.23	U (38) = 13.50; Z = 5.03	*
Mid level stamen (mm)	5.02 ± 0.35	3.40 ± 0.38	t (38) = 13.67	*
Petal surface area (cm²)	0.49 ± 0.11	0.42 ± 0.11	t (34) = 1.82	ns
Leaf surface area (cm²)	1.94 ± 0.67	1.66 ± 0.67	U (38) = 149.50; Z = 1.35	ns
Total				
Number of flowers	5.76 ± 4.16	4.06 ± 2.78	U (158) = 2346.50; Z = 2.91	*
Total dry mass (g)	0.27 ± 0.40	0.16 ± 0.14	U (152) = 2325.50; Z = 2.30	*
Style length (mm)	7.47 ± 0.57	5.30 ± 1.51	U (158) = 495.00; Z = 9.22	*
Mid level stamen (mm)	4.91 ± 0.35	3.22 ± 0.35	t (158) = 29.90	*
Petal surface area (cm²)	0.50 ± 0.14	0.43 ± 0.11	t (154) = 3.62	*
Leaf surface area (cm²)	1.90 ± 0.89	1.46 ± 0.64	U (158) = 2257.50; Z = 3.21	*
Oxalis nidulans				
Kogelberg Nature Reserve				
Number of flowers	1.90 ± 1.16	2.2 ± 1.23	U (38) = 171.00; Z = –0.77	ns
Total dry mass (g)	0.02 ± 0.01	0.02 ± 0.01	t (34) = 0.76	ns
Style length (mm)	8.63 ± 0.34	7.08 ± 0.65	t (38) = 9.31	*
Mid level stamen (mm)	7.05 ± 0.39	5.37 ± 0.48	t (38) = 12.01	*
Petal surface area (cm²)	1.02 ± 0.25	0.59 ± 0.10	t (38) = 7.00	*
Leaf surface area (cm²)	0.93 ± 0.36	0.87 ± 0.28	t (38) = 0.63	ns

Character	Healthy	Diseased	Test value (df)	P
Oxalis lanata				
Paradyskloof				
Number of flowers	5.70 ± 5.75	6.45 ± 5.22	U (38) = 176.5; Z = –0.62	ns
Total dry mass (g)	0.26 ± 0.14	0.18 ± 0.12	U (38) = 100.50; Z = 2.67	*
Style length (mm)	7.24 ± 0.30	5.49 ± 0.78	U (38) = 28.00; Z = 4.63	*
Mid level stamen (mm)	5.56 ± 0.25	4.41 ± 0.56	U (38) = 7.00; Z = 5.20	*
Petal surface area (cm²)	1.21 ± 0.18	1.00 ± 0.24	t (38) = 3.09	*
Leaf surface area (cm²)	2.37 ± 0.73	1.29 ± 0.42	U (38) = 26.00; Z = 4.69	*
Brandwacht				
Number of flowers	13.20 ± 5.61	6.70 ± 2.69	t (38) = 4.66	*
Total dry mass (g)	0.80 ± 0.33	0.36 ± 0.19	U (38) = 48.50; Z = 4.08	*
Style length (mm)	7.13 ± 0.69	4.64 ± 0.51	U (38) = 0.00; Z = 5.39	*
Mid level stamen (mm)	5.11 ± 0.24	4.14 ± 0.51	t (38) = 7.56	*
Petal surface area (cm²)	1.23 ± 0.18	0.83 ± 0.16	U (38) = 23.00; Z = 4.77	*
Leaf surface area (cm²)	1.92 ± 0.42	1.18 ± 0.30	U (38) = 24.50; Z = 4.73	*
Stellenbosch Mountain				
Number of flowers	8.95 ± 4.37	4.75 ± 3.36	t (38) = 3.40	*
Total dry mass (g)	0.49 ± 0.23	0.25 ± 0.11	U (38) = 78.00; Z = 3.28	*
Style length (mm)	7.27 ± 0.40	5.09 ± 0.40	t (38) = 12.97	*
Mid level stamen (mm)	5.17 ± 0.44	4.81 ± 0.67	t (38) = 2.00	*
Petal surface area (cm²)	0.90 ± 0.15	0.60 ± 0.11	t (38) = 7.29	*
Leaf surface area (cm²)	1.82 ± 0.44	1.28 ± 0.51	U (38) = 98.50; Z = 2.73	*
Constantia Neck				
Number of flowers	3.80 ± 1.90	5.05 ± 2.25	U (38) = 127.50; Z = –1.94	ns
Total dry mass (g)	0.11 ± 0.03	0.08 ± 0.02	U (38) = 103.00; Z = 2.61	*
Style length (mm)	7.99 ± 0.47	6.32 ± 1.00	U (38) = 22.00; Z = 4.80	*
Mid level stamen (mm)	5.19 ± 0.38	3.51 ± 0.27	t (38) = 15.89	*
Petal surface area (cm²)	1.08 ± 0.19	0.83 ± 0.13	t (38) = 4.71	*
Leaf surface area (cm²)	1.17 ± 0.41	0.98 ± 0.36	U (38) = 150.00; Z = 1.33	ns
Jonkershoek				
Number of flowers	2.75 ± 1.55	2.85 ± 1.56	U (38) = 195.50; Z = –0.10	ns
Total dry mass (g)	0.22 ± 0.08	0.16 ± 0.12	U (38) = 108.00; Z = 2.47	ns
Style length (mm)	7.08 ± 0.31	4.16 ± 0.86	U (38) = 1.00; Z = 5.36	*
Mid level stamen (mm)	5.09 ± 0.33	3.32 ± 0.61	U (38) = 15.00; Z = 4.99	*
Petal surface area (cm²)	0.61 ± 0.12	0.43 ± 0.15	U (38) = 76.00; Z = 3.34	*
Leaf surface area (cm²)	1.17 ± 0.44	1.04 ± 0.43	U (38) = 166.00; Z = 0.90	*
Total				
Number of flowers	6.88 ± 5.63	5.16 ± 3.49	t (198) = 2.59	*
Total dry mass (g)	0.37 ± 0.31	0.21 ± 0.15	t (198) = 4.78	*
Style length (mm)	7.34 ± 0.56	5.14 ± 1.06	t (198) = 18.28	*
Mid level stamen (mm)	5.23 ± 0.38	4.04 ± 0.77	t (198) = 13.79	*
Petal surface area (cm²)	1.01 ± 0.28	0.74 ± 0.27	t (198) = 7.06	*
Leaf surface area (cm²)	1.70 ± 0.68	1.13 ± 0.43	t (198) = 6.65	*

Some form of anthropogenic disturbance was noted at most infected populations. Such disturbance could be detrimental to the population if fungal spores were air borne and could enter new hosts through vegetative tissue (e.g. *M. violaceum* s. l.) or soil borne and infected new seedlings (e.g. *Tilletia contraversa* J. G. Kühn s. l.) (Ngugi & Scherm 2006). Such threats are real, given that many *Oxalis* species are exendospermous, which causes seeds to germinate within the same flowering season as their production (Salter 1944). Diseased adult plants and new seedlings will thus be sympatric in time and space. Increased shade cover observed at some of the diseased populations may contribute to decreased spore desiccation, which increases chances of spore viability once it reaches a new host growing in the shade. A study on flower smut fungus *Sporisorium amphilophis* (Syd.) Langdon & Full. on *Bothriochloa macra* (Steud.) S. T. Blake suggested that populations located in disturbed roadside habitats were more likely to be infected than those in preserved areas (García-Guzmán *et al.* 1996). The same may hold true here, as the majority of diseased populations occurred along roadsides.

Tab. 3. Reproductive effect of *Thecaphora capensis* on *Oxalis incarnata* and *Oxalis lanata* from various populations. Results are reported as median ± standard deviation. Different superscript letters indicate difference in significance between ranked means for the individual comparisons. Significance is reported (*) when $P \leq 0.05$. df = degrees of freedom. ns = not significant.

	Natural seed set	Diseased individuals	Healthy individuals	Spores and pollen	Test value (df)	P
Oxalis incarnata						
Cecilia Forest	0.18 ± 0.40	0	0.33 ± 0.81	0	H (3) = 4.52	ns
Homtini Pass	1.83 ± 2.03^{a}	0^{b}	0.31 ± 0.79^{ab}	1.38 ± 1.78^{a}	H (3) = 20.57	*
Gouna	2.50 ± 1.57^{a}	0^{b}	1.72 ± 1.67^{a}	1.46 ± 1.18^{a}	H (3) = 29.87	*
Phantom Pass	0.88 ± 1.49	0	1.37 ± 1.96	1.05 ± 2.01	H (3) = 7.48	ns
Total	1.44 ± 1.74^{a}	0^{b}	0.96 ± 1.53^{a}	1.07 ± 1.63^{a}	H (3) = 44.92	*
Oxalis lanata						
Paradyskloof	3.31 ± 4.39^{ab}	0^{a}	4.94 ± 5.60^{b}	4.15 ± 4.76^{ab}	H (3) = 13.45	*
Brandwacht	0^{a}	0.05 ± 0.22^{a}	10.20 ± 6.05^{b}	3.25 ± 4.45^{a}	H (3) = 49.30	*
Stellenbosch Mountain	1.35 ± 4.98^{ab}	0^{a}	6.45 ± 6.75^{bc}	10.50 ± 9.41^{c}	H (3) = 26.97	*
Constantia Neck	1.85 ± 5.57^{a}	0.20 ± 0.89^{a}	8.90 ± 7.31^{b}	9.00 ± 7.70^{b}	H (3) = 32.90	*
Jonkershoek	7.90 ± 5.59^{a}	0^{b}	7.84 ± 6.14^{a}	6.90 ± 5.12^{a}	H (3) = 29.58	*
Total	2.90 ± 5.31^{a}	0.05 ± 0.41^{b}	7.75 ± 6.55^{c}	6.64 ± 6.90^{c}	H (3) = 119.21	*

Although insects have been identified as carriers of *Thecaphora capensis* spores (Curran *et al.* 2009), the specific site of infection has not yet been identified. However, since the fungus has evolved to produce spores in such a specialised niche, it can be assumed that flower-visiting insects play a primary role in fungal transmission and that flowers are a major site for infection. During our investigations we often observed Cape honeybees (*Apis mellifera capensis*) visiting flowers of diseased plants and presume that these may play a large role in fungal transmission. Future studies should aim to

determine the role of flower visitors in fungal transmission and the main sites of new infections.

In the southwestern GCFR, natural communities often house between three and eight sympatric species of *Oxalis* (De Jager *et al*. 2011). Sympatric *Oxalis* species with no signs of infection were found in all localities where individuals of a species were found to be infected with *Thecaphora capensis*. No localities contained more than one infected host *Oxalis* species. This was a surprising find if it is assumed that several phylogenetically unrelated *Oxalis* species are susceptible to infection by *T. capensis* as suggested by the spread of hosts across the *Oxalis* phylogeny. However, restricted pollinator movement between conspecific *Oxalis* species in *Oxalis* communities may enforce these patterns. De Jager *et al*. (2011) showed that pollinators of *Oxalis*, specifically individual honeybees, were unlikely to alternate between sympatric *Oxalis* species. However, other floral traits, including flower size, may also influence pollinator preferences when flower colours of co-occurring *Oxalis* species are similar. These pollinator preferences could ultimately lead to restrictions on the movement of *T. capensis* between co-occurring hosts. Alternatively, host-pathogen co-evolution may restrict infection to certain genotypes of *T. capensis* and specific *Oxalis* hosts.

Morphologically, *T. capensis* induce negative effects on diseased *O. lanata* plants that included various flower abnormalities. These abnormalities have not been observed in other diseased *Oxalis* species. *Microbotryum violaceum* s. l. has also been shown to affect some hosts by inducing stunted growth and asymmetrical flowers with elongated petal claws (Baker 1947). Diseased plants of *O. incarnata*, *O. lanata* and *O. nidulans* were easily identifiable within a population, as they visibly appeared sickly, bearing smaller petals and leaves. This reduction in size may be attributed to the utilization of host resources (Kover 2000, Ngugi & Scherm 2006) by *T. capensis* for production of its own spores. Our results contrast those obtained in a study of *M. violaceum* on *Silene acaulis* L. (= *M. silenes-acaulis* M. Lutz, Piątek & Kemler, Lutz *et al*. 2008) that showed a significant increase in plant size after infection (Marr 1997). However, it is similar to this system in that, except for a single population of *O. lanata*, fungal infection did not lead to the production of more flowers. This, in turn, contrasts to results obtained in a study of the *M. violaceum* s. l.-*S. latifolia* system (= *M. lychnidis-dioicae* (DC.) G. Deml & Oberw., Deml & Oberwinkler 1982), in which Alexander (1987) and Alexander & Maltby (1990) observed increased flower production following infection. Infection by *T. capensis* decreases the size of individual flowers, as was found in the *M. violaceum*-*S. latifolia* system (Alexander & Maltby 1990). Production of shorter styles and stamens in diseased flowers may also result from resource allocation towards the fungus. Longer stamens with anthers containing fungal spores may result in spore losses through wind dispersal, which may explain the shorter stamens of infected flowers. Shorter stamens may also be advantageous to *T. capensis* in terms of successful insect vectoring, since pollinators would have to probe deeper into flowers to reach their potential pollen reward and collect more spores in the process.

Infected *O. incarnata* and *O. lanata* flowers were rendered virtually sterile. These flowers now only function as fungal reproduction organs, and are no longer capable of seed production. Infected hosts are therefore totally reliant on clonal reproduction through bulbil formation (Salter 1944). However, since clumps of diseased plants in *O. lanata* populations are often of a single floral morph, the fungus is likely able to spread through plant clonal reproduction (Curran *et al.* 2009). Diseased individuals thus seem to be permanently removed from the gene pool. Importantly, all infected populations still contained some healthy plants that produced healthy flowers and seeds. Such plants could both persist and produce healthy progeny in an infected population, but further work is needed to determine whether this persistence is the result of chance or genetic resistance to infection. The effect of host and pathogen genetic composition on disease spread should thus be considered in future studies.

Healthy flowers that received both fungal spores and legitimate pollen displayed no variation in the number of seeds produced compared to healthy flowers that received only legitimate pollen. Opposite results were shown by Marr (1997), where healthy flowers of *S. acaulis* that received both *M. violaceum* s. l. spores and healthy pollen had reduced seed set. The reduced seed set was ascribed to poorer pollen germination in the presence of *M. violaceum* s. l. spores due to chemical interference, as the presence of foreign pollen had no negative influence (Marr 1998). *Oxalis* species that receive mixtures of conspecific and foreign congeneric pollen are known to produce less seeds than flowers receiving only legitimate pollen (De Jager *et al.* 2011). The lack of influence on seed set of healthy *Oxalis* inoculated with a pollen/spore mixture is thus interesting, but tricky to interpret. It is possible that, as healthy plants in diseased populations were used for these studies, plants may have acquired natural resistance against *T. capensis* obtained through natural selection imposed by disease presence. This seemingly unaffected production of seeds by flowers that received both legitimate pollen and *T. capensis* spores may also be ascribed to the time required for the fungus to infect the plant, since it has been suggested that fungal infections may take two years (Alexander & Antonovics 1988), with normal seed production the first year after infection. Future studies should thus explore the effect of spore presence on seed set using plants from healthy populations.

In this study we have contributed to a growing base of knowledge on the ecology of *T. capensis* on *Oxalis* (Roets *et al.* 2008, Curran *et al.* 2009) and provide further insight into this smut-fungus-plant interaction in general. We have shown that the influence on host morphology and reproductive success may differ between congeneric hosts. In order to identify overall patterns, it is important to include not only numerous species in investigations for general host-pathogen dynamics studies, but also to include multiple populations of these species. The number of known host species for *Thecaphora capensis* has been increased and we suggest that further monitoring for this fungus in the GCFR is needed. As this fungus has drastic negative effects on host morphology and reproduction, it is essential to identify fur-

ther infections and to monitor the extent of these infections into the future. This is especially true when planning for the future conservation of the numerous red listed species in the genus, since more than a third of all southern African *Oxalis* species are listed as either rare or endangered (Raimondo 2011).

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