Influence of feeding-location on nutritional quality for cochineal insects (Homoptera: Dactylopiidae) on *Opuntia ficus-indica* (Cactaceae)

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Abstract

The cochineal insect *Dactylopius opuntiae* has been an extremely successful biocontrol agent of the cactus weed *Opuntia ficus-indica.* Its resounding success has allowed us to take a closer look at how the relationships between biocontrol agents and their host plants are maintained. The prickly pear, *O. ficus-indica*, is the sole source of food for the cochineal insect and provides it with refuge and shelter. Wind, sun and particularly rain leave the cochineal insects vulnerable to predators as their protective waxy coating is eroded by the elements. The behaviour of the insects to settle on the sheltered side of cactus cladodes is not unexpected when one takes the aforementioned stresses into account. One curious fact that has arisen from this is that the insects that do settle on the sheltered side of the cladode are larger and more fecund than those that settle on the exposed surface of the cladode. The nutritional quality of the plant was investigated as a possible explanation for the disparity in size. Phloem sap was collected from the severed stylets of cochineal insects and analysed for polyphenols (which inhibit growth) and amino acids (responsible for growth). The indication from the polyphenol analysis was that it was not responsible for inhibiting growth why ? in cochineal insects. Amino acid analysis from two different cladodes gave conflicting results and no definite conclusions could be drawn from these. Increasing the number of samples analysed will give us a better idea of any trends that could possibly exist. It is certain that there is still much to be discovered in the way of insect-plant interactions and future studies in this field could yield some potentially remarkable findings

Introduction

Biological control is the use of natural enemies to reduce the growth or reproductive potential of an alien, invasive plant (Klein 2002a, 2002b). Plants when introduced to a new region often become invasive in the absence of any natural enemies. This gives the alien plants a competitive advantage over indigenous plants that have their own natural enemies. Thus, biological control is the introduction one/more of an alien plant's natural enem~~y~~ies to its new habitat in an attempt to remove any competitive advantage that the alien plant has over the natural vegetation (Klein 2002a, 2002b). Natural enemies introduced for the purposes of biological control are called biocontrol agents.

The introduction of alien organisms as biocontrol agents has become a highly specialised science. Introduction of a organism to a new area is associated with many potential hazards. Alien organisms can wreak havoc on the indigenous flora and fauna and in a number of cases have driven many indigenous plants or animals to extinction (Klein 2002b). It is because of this potential risk that the government SA ?, before granting permission to release biological control agents, has implemented strict rules and regulations. Most importantly, potential agents have to prove sufficiently host-specific before permission to be released can be sought. To be considered host-specific, the biocontrol agent on trial has to be either monophagous (feed on a single plant species) or to feed on a host plant or plants that do not occur in South Africa and surrounding countries, either as indigenous plants or introduced crop plants (Klein 2002b).

However, despite being extremely effective, biocontrol agents do not completely eliminate populations of their host plant. Instead they can be expected to reduce the density of alien vegetation to a more manageable level or to reduce the reproductive potential of alien plants. A biological control project is considered a complete success if no other control measures are required to keep the invasive under control. The fact that some part of the host plant population will survive ensures that the biocontrol agent will not become extinct as a result of lack of food. Biocontrol can therefore be regarded as a sustainable control method (Klein 2002b).

Biological control has a number of other advantages over other methods of control. ~~1)~~ It
1) causes no pollution and only affects the invasive plant, making it environmentally friendly. 2) As mentioned above it is self-sustaining so reintroduction is not necessary. 3) Since we are using a naturally occurring organism it is very cost-effective and 4) by not eliminating vast stands of alien plant at once it allows the indigenous vegetation to gradually replace the invasive plants without possibility of other invaders establishing themselves (Klein 2002b).

Some of the first and most successful biocontrol projects undertaken in South Africa have been against a number of pest cactus species. The first biological weed control project in South Africa was against the smooth prickly pear (*Opuntia monacantha*) in 1913 (Klein 2002c, 2002e). Cochineal insects (*Dactylopius spp.*) no itali are the primary biological control agents used against this pest cactus and against all other cactus species. Probably one of the most dramatic successes of biological control has been the regulation of the prickly pear (*Opuntia ficus-indica*).

The prickly pear, *Opuntia ficus-indica* (L.) Miller

The prickly pear, *Opuntia ficus-indica*, is an invasive pest plant indigenous to Central America that has flourished in South Africa for more than 200 years (Annecke & Moran

1978; Zimmerman & Moran 1991; Githure *et al.* 1999). Despite attempts at mechanical and herbicidal control dating back to the 1890s *O. ficus-indica* has continued to thrive (Annecke & Moran 1978). By 1942 about 900 000 ha of land was infested, the heaviest concentrations occurring in the eastern Cape Province and the Karoo (Annecke & Moran 1978; Zimmerman & Moran 1991).

O. ficus-indica is a tall tree cactus that can reach heights of greater than 4 metres with the main stems become woody with age (Annecke & Moran 1978). The plants form impenetrable thickets covering wide areas, overwhelming indigenous vegetation and disrupting agricultural activities (Annecke & Moran 1978). The cactus cladodes (modified stems forming leafpads) have numerous sharp spines and glochids scattered over the surface. Livestock eat the fruit and cladodes of *O. ficus-indica* with the spines and glochids potentially leading to severe or fatal gastro-intestinal disorders (Annecke & Moran 1978).

Despite the status of a pest weed there are certain situations where *O. ficus-indica* is exploited for its benefits. The fruits that the plants produce are succulent and often sold along the roadside, particularly in the eastern Cape. Cladodes of the prickly pear have been used to a limited extent as fodder despite requiring significant effort to remove the thorns and providing a relatively nutritionally poor food source (Annecke & Moran 1978). Other varieties of *O. ficus-indica* having no spines or a few short ones (collectively known as the 'Burbank' spineless cacti) have been cultivated, particularly in the Karoo, as insurance fodder for stock during droughts (Annecke & Moran 1978; Githure *et al.* 1999).

The biological control campaign against *O. ficus-indica* was launched in the 1930s when the benefits derived from the plants were minimal compared to the losses incurred (Githure *et al.* 1999).

The cochineal insect, *Dactylopius opuntiae* (Cockerell)

Dactylopius opuntiae is one of nine species of cochineal insects in the monogeneric family Dactylopiidae (Hemiptera), all of which feed on cactaceous plants and almost exclusively on the genus *Opuntia* (De Lotto 1974).

Cochineal insects live in colonies or clusters of individuals in various stages of development on the surface of the cactus plants. They secrete white waxy filaments that cover their bodies. The female first instar nymphs (known as crawlers) are mobile and constitute the dispersal phase of the life cycle along with the winged adult males, which disperse through flight (Moran & Cobby 1979). Once the female crawlers have colonised a new host plant or feeding site (which may take up to ten days) they insert their mouthparts into the plant tissue and begin to feed. They remain sessile and continue to feed on the same plant for the rest of their lives (Moran *et al.* 1987; Moran & Cobby 1979). After initiating feeding, the female nymphs start to secrete waxy, woolly filaments that protect their bodies from heat, cold and predation by coccinellid beetle predators (ladybirds) (Annecke & Moran 1978; Moran *et al.* 1987). The body fluids of female cochineal insects also contain high concentrations of carminic acid, seemingly to deter parasites (Klein 2002d).

Dactylopius opuntiae has played a central role in the successful biological control campaign against *Opuntia ficus-indica* in South Africa (Moran & Cobby 1979; Moran *et al.* 1987; Githure *et al.* 1999; Volchansky *et al.* 1999). It has been so successful that almost 90% of the original 900 000 ha infested have been reclaimed for pastoral use (Annecke & Moran 1978; Zimmerman & Moran 1991). Today only small dense populations can be found at the coastal areas of the eastern Cape and isolated pockets in the Karoo and Transvaal (Zimmerman & Moran 1991). The control of *O. ficus-indica* has meant that the spineless varieties can be cultivated as a stock feed providing precautions have been taken against *Dactylopius opuntiae.* The remaining prickly pear infestations in South Africa have enormous potential as host plants for the commercial production of *Dactylopius coccus* O. Costa, also known as the "true" cochineal. *Dactylopius coccus* are rich source of carminic dye used in foodstuffs, cosmetics and pharmaceutical products (Zimmerman & Moran 1991).

Factors affecting the efficacy of cochineal insects

Annecke and Moran (1978) noted that cochineal insects predominate on the rain- and wind-sheltered sides of the cladodes. It was suggested that this be due to increased vulnerability to coccinellid predators on the exposed surface, where the cochineal's protective wax coating is eroded by wind and rain. Rain and wind was also thought to prevent immature stages from settling on exposed surfaces and that final settling positions are influenced by negative phototrophic responses (Annecke & Moran 1978). The e~~a~~ffects of rainfall on the effectiveness of *D. opuntiae* has been quantified and documented by Moran and Hoffman (1987) and Moran *et al.* (1987). The negative phototactic response of crawlers was also noted by Moran *et al.* (1987).

The climatic conditions mentioned above could account for the predominance of cochineal insects on the sheltered sides of cladodes. Females on the sheltered surface of the cladode are generally larger at maturity than the females on the exposed surface (J.H. Hoffmann, pers. com. 2002). The number of progeny produced by females is positively correlated with body mass inferring direct fitness benefit from inhabiting the sheltered surfaces (Moran & Cobby 1979; Volchansky *et al.* 1999). The difference in size and fecundity can't be sufficiently explained by climatic conditions alone, suggesting some other factor affecting growth of the cochineal insects. There may be one of two factors influencing their growth; either there is something in their food source directly inhibiting their growth or the nutritional quality of the food is sufficiently different to cause the observed differences.

Light as a factor affecting food quality

After noting that the crawlers reacted negatively to light when settling it seemed logical to expect that light may also have something to do with inhibited growth. Indeed Waterman and Mole (1989) found that a number of studies related changes in incident light to production in phenolic allelochemics. – in which species? There was an emergent pattern that individuals within a species, or parts of an individual exposed to higher light intensities, are likely to respond by producing more secondary metabolites than congeners in more shaded environments. Reductions in growth rate of insects have been found to correlate with increasing content of phenolics (Reimer & Whittaker 1989). Homopterans are particularly sensitive to phenolics and negative correlations between phenolic content of leaves and susceptibility to aphids have been shown (Reimer & Whittaker 1989). While plants exposed to a high intensity of light produce more secondary metabolites, Collinge and Louda (1988) demonstrated that artificially shaded plants had a higher level of available nitrogen and total amino acids. This finding may explain the differences observed in *D. opuntiae,* as growth and reproduction of phytophagous insects is influenced by the quantity and quality of the proteins and amino acids in their food (McNeill & Southwood 1978).

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Nitrogen and Amino Acids

There are ten amino acids generally considered essential in insect diets for normal growth: arginine, lysine, leucine, isoleucine, tryptophan, histidine, phenylalanine, methionine, valine and threonine (McNeill & Southwood 1978; Ishaaya 1986). The importance of organic nitrogen for normal insect growth and reproductive success cannot be overemphasised as the growth efficiency of a variety of insects is closely related with plant nitrogen content, a correlate of protein content (Reimer & Whittaker 1989; Schoonhoven *et al.* 1998, Mattson 1980). As the nitrogen content of their food increases, insects become more efficient in converting plant material into body tissue (Schoonhoven *et al.* 1998). Total nitrogen levels of a plant can give misleading results and is frequently a poor index of its nutritional value as not all the nitrogen may be available to the insect (McNeill & Southwood 1978; Schoonhoven *et al.* 1998). Phloem feeding species are in a more privileged position than chewing insects, because nearly all nitrogen-containing compounds in phloem sap can be utilised and phloem sap contains 10 to 100 times more nitrogen than xylem sap (Mattson 1980; Schoonhoven *et al.* 1998). The nitrogenous constituents of phloem include mostly amides, free amino acids and perhaps some peptides (Montllor 1989).

Aims

there

From the literature it is evident that are two possible explanations for the greater size of cochineal insects on sheltered sides of cladodes compared to cochineal insects on the exposed sides. We can therefore make two hypotheses:

1) More polyphenols are produced on the exposed surfaces where photosynthesis takes place compared with the sheltered surfaces therefore inhibiting the growth of the insect.
2) The nitrogen content and more specifically the amino acid constituents on the sheltered surface are more plentiful and available for the insect to utilise for growth.

This study will attempt to show if either, or both, of the hypotheses are correct. This study is important for creating a clearer picture of the relationship between *D. opuntiae* and its host *O. ficus-indica*. Understanding the relationships between a biological control agent and its host plant is of vital importance in effective management. By learning how plant defences affect the success of this dramatically successful biological control agent we can gain some insight into the relationships between other less successful biological control agents and their host plants. This will allow us to determine why particular insects are not successful and guide us in making more productive decisions in boosting alien control.

Materials and methods

The effect of feeding position on food quality was determined using cochineal females reared on detached cladodes and potted plants of *Opuntia ficus-indica*. Five cladodes were removed from potted *O. ficus-indica* plants maintained in a greenhouse. Each detached cladode was placed horizontally on the heads of three pins that had been inserted into a polystyrene block. This provided the insects with access to almost the entire surface of the cladode and placed minimal restrictions on where they could settle and feed. Even though cochineal insects can be reared as effectively on detached cladodes as on rooted plants (Moran & Cobby 1979), one set of females was reared on a potted *O. ficus-indica* plant to enable comparison with the those reared on the detached cladodes. The cladodes with insects were maintained in a controlled environment room at 28 ± 2 °C during daylight 24 ± 2 °C during night with fluorescent lights on a 12-h daylight cycle to provide an environment suitable to the insects (Moran & Cobby 1979).

Each of the detached cladodes, and the potted plant, were 'seeded' with approximately 30 neonate nymphs, transferred individually with a fine paintbrush onto each experimental cladode. Thirty crawlers were placed on each cladode to ensure sufficient food for each insect and to prevent overcrowding, which could have stunted the growth of females (Moran & Cobby 1979). The insects were left to develop for approximately 40 days until the females reached maturity and started producing offspring. At this stage they were removed from the cladode, by severing their stylets, and weighed. The significance in the difference of the masses of female cochineal insects found on alternate sides of a cladode was calculated using an independent t-test for each cladode (Zar 1999). The stylets were cut using a fine razor blade viewed under a dissecting microscope (Wang & Nobel 1995).

Phloem collection

The area around the protruding stylets was washed with 70% ethanol to ensure no impurities were collected when obtaining the phloem. Exuding phloem sap was collected from the severed ends by inserting the protruding portion of the stylets into one end of a 2-μl capillary tube. The tubes were examined every hour and removed when either the tubes were full or flow had ceased. The phloem was then blown out of the capillary tube into a plastic eppendorff tube and frozen.

Polyphenol analysis

High Performance Liquid Chromatography (HPLC) was used to analyse polyphenols in *O. ficus-indica* exudates . 3 µl of phloem from the original extract was diluted in 20 µl of 70% methanol and 10 µl was injected for processing with HPLC. A method was developed using a Spherisorb ODS 2 (5 micron) column (250 x 4.5 mm) and acetonitrile gradient elution. where was this done ?

Solution A = 0.1% TFA water (trifluroacetic acid)

Solution B = 100% acetonitrile

Gradient:	time	%B	flow (ml/min)
	0	0	0.7
	5	0	0.7
	55	100	0.7
	60	100	0.7
	65	0	0.7
	66	0	0.1
	67	0	stop

Amino acid analysis

Gas-phase Acid Hydrolysis

2µg of the protein sample was placed in hydrolysis tubes and dried down in a vacuum. The dried down samples were placed in a gas phase hydrolysis vessel, to which 6M hydrochloric acid and 0.2% phenol was added to act as an antioxidant. This was then hydrolysed for 24 hours at 110°C. After 24 hours the sample was once again dried down using a vacuum and 50μl of 0.2 mol. citrate, pH 2.2 (sample application buffer) was added (Kencht & Chang 1986).

Amino acid analysis

A cation ion exchange resin was injected through a column (0.4 X 25 cm). Post-column derivatization of amino aids was done using O-Phthaldehyde (OPA) (Klapper 1982).

Results

The mass of gravid females on the exposed surfaces of cladodes versus the sheltered surfaces:

There was a significant difference on all of the cladodes (t-test: $t < -2.9$, df = 19, $p < 0.01$) with the females on the sheltered surfaces being larger than those on the exposed surfaces (*Table 1*).

Table 1. The mean masses of female cochineal insects, showing the differences between the exposed and sheltered sides, of seven cladodes (significance level $p<0.05$).

Cladode type	number	Mean Mass(g)		t-value	d.f.	p
		Top	Bottom			
Detached:	1	0.019	0.025	-3.3	26	0.003
	2	0.012	0.017	-3.4	25	0.002
	3	0.020	0.046	-6.4	20	0.000
	4	0.027	0.036	-2.9	19	0.010
	5	0.009	0.029	-12.2	25	0.000
Potted:	1	0.025	0.038	-5.3	24	0.000
	2	0.026	0.037	-4.0	24	0.001

Polyphenol Content

The content of polyphenols in the phloem extracts of the sheltered and exposed sides of a detached cladode is indicated by the HPLC elution profiles in *Figures 1a and 1b* respectively

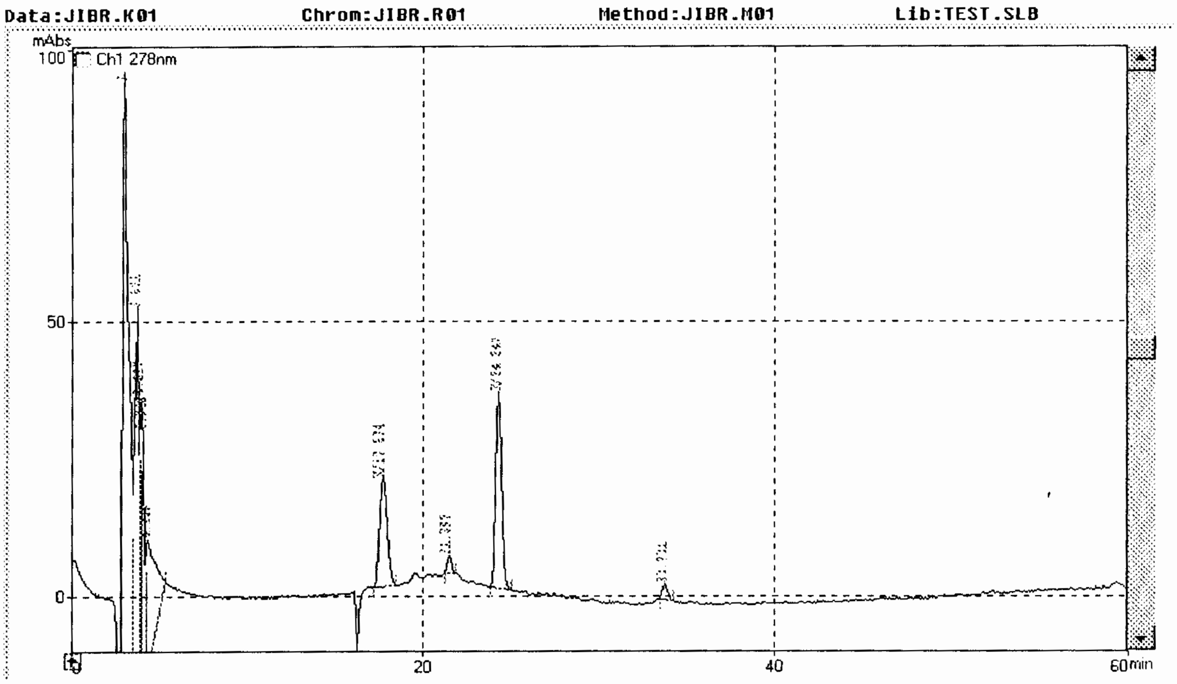

Figure 1a. The HPLC elution profile of polyphenols in the phloem collected from the sheltered surface of cladode 1.

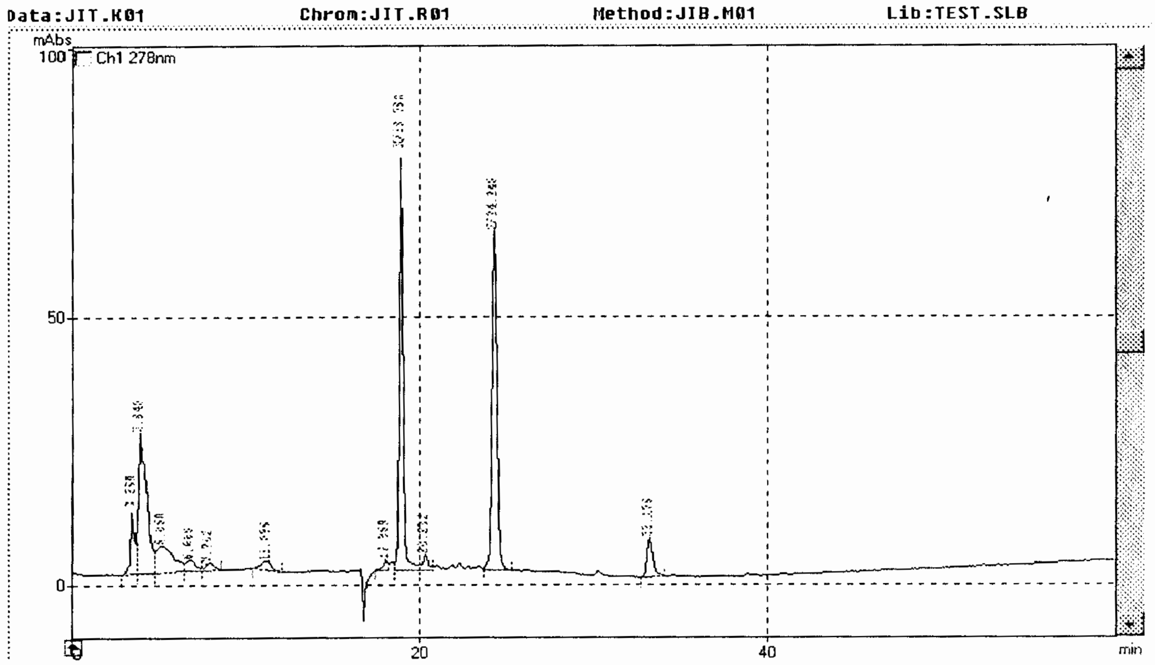

Figure 1b. The HPLC elution profile of polyphenols in the phloem collected from the exposed surface of cladode 1.

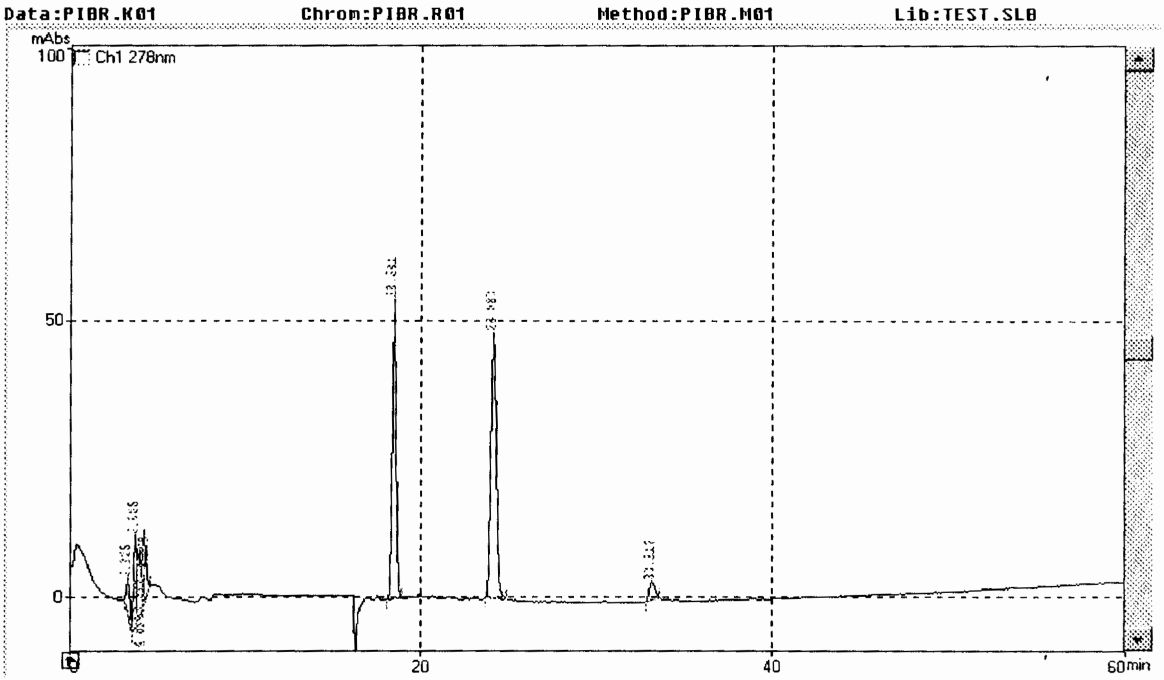

Figure 2a. The HPLC elution profile of polyphenols in the phloem collected from the sheltered surface of a potted cladode.

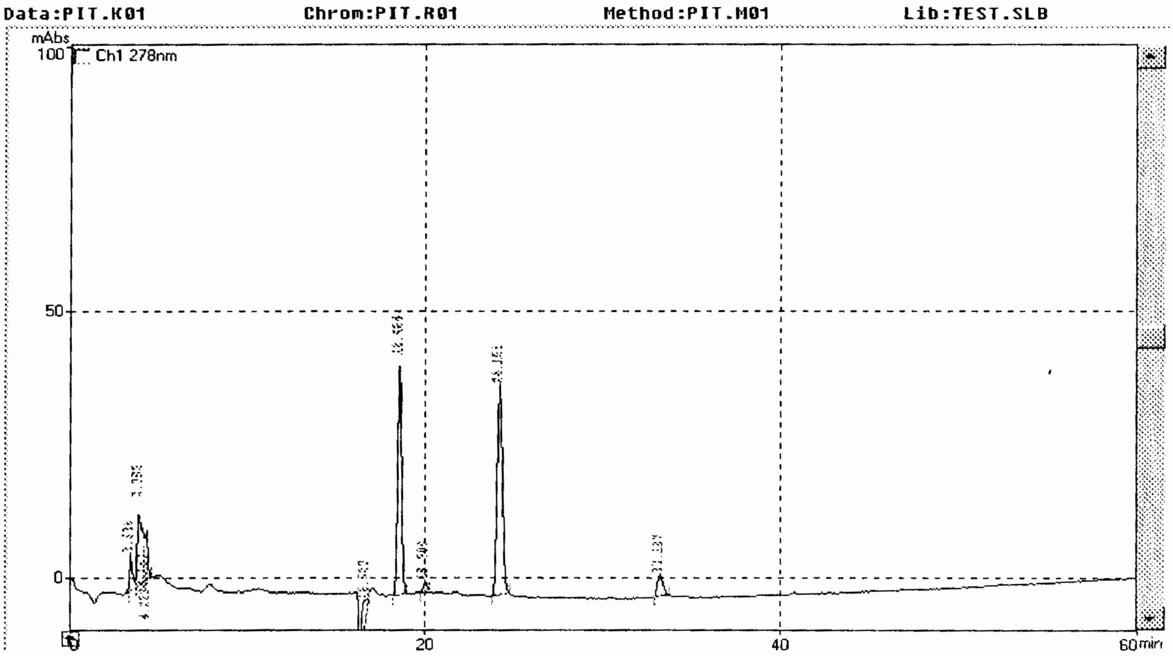

Figure 2b. The HPLC elution profile of polyphenols in the phloem collected from the exposed surface of a potted cladode.

The phloem solution is run through a column for an hour and the different peaks observed represent different compounds and their respective polarities over time. *Figure 1a* shows a large peak near the beginning of the run probably caused by a polar compound such as amino acids or base metabolites, which are hydrophilic.

Figure 1a continued... There are four distinct peaks occurring between 17 and 40 minutes showing the presence of four aromatic polyphenols.

Figure 1b shows a similar peak of polar compounds at the beginning, but to a much lesser degree. The same four polyphenol peaks are observed as in *figure 1a,* but the peaks are more pronounced. The proportion of compound 1 to compound 3 is greater than in the first elution profile.

Figures 2a and *2b* show the polyphenol profile of the phloem solution obtained from the sheltered side and the exposed side of a potted cladode. The polar peaks at the beginning of the run are very similar in shape and area to each other. The polyphenol profiles are almost identical, the only noticeable difference being the total amount of polyphenols present. There is slightly more present in the phloem extracted from the sheltered side of the plant.

Amino Acids

The total amount of amino acids and the constituent amino acids present in the phloem on either side of two cladodes is presented in *Table 2.* The exposed surface of the detached cladode had more total amino acids present than the sheltered surface. The exact opposite is found on the potted cladode where almost twice as many amino acids are found on the exposed side than on the sheltered side.

Discussion

The masses of females on sheltered sides of cladodes are significantly greater than those found on the exposed sides. This supports the findings of Hoffmann (pers. com. 2002) that females on the lower surface of the cladode are generally larger at maturity than those on the upper surface and warrants investigation into the cause of this phenomenon.

The analysis of polyphenols showed that there was almost no difference between the compounds found on either side of the cladode. The same phenolic compounds were present on all of the surfaces indicating light has no affect of the type of polyphenols produced. Only slight changes in amount were noted, but there was inconsistency between the different cladodes with regard to which side of the plant contained more of the polyphenol. It is well documented that insect growth is hampered by the presence of polyphenols (McNeill & Southwood 1978; Reimer & Whittaker 1989) however, it does not appear that in the case of *O. ficus-indica,* light has any affect on the production of polyphenols as found in other plants (Collinge & Louda 1988; Waterman and Mole 1989).

Light has been shown to have an affect on the nitrogen and amino acid content of numerous plant species (Collinge & Louda 1988). The results in this study show two conflicting ratios of amino acids found on the exposed and sheltered surfaces of two different cladodes. The potted cladode had more than twice the amount of amino acids on the sheltered side, which would explain the increased growth of the female cochineal insects on this side of the cladode. Contrary to this trend, the detached cladode shows a marked increase in amino acids on the exposed surface. A truer indication of nutrients available to the insect in the wild is expected to be found in the cactus that is potted. The detached cladode might possibly be using certain compounds, which normally the insect would feed on, to protect itself from desiccation. This is supported by the fact that the cochineal insects on the potted cladodes were found to be significantly larger than those on the detached cladodes (ANOVA $F_{(2, 175)} = 5.87$, $p < 0.02$).

No concrete conclusions can be drawn from these preliminary results, but results do appear to suggest polyphenols are not responsible for the stunted growth observed in females settling on the exposed surfaces of cladodes. Likewise, there is no solid evidence the amino acids are responsible for disparity in insect growth. Perhaps with a larger sample size a more distinct pattern may emerge, which could be used to explain the findings. Further studies may benefit from rearing more cochineal colonies on potted plants rather than detached cladodes when analysing plant nutrients.

Further research

There are a number of other studies, which might provide some insight on this predicament. The flow rate of phloem exuding from the mouthparts of the cochineal insect could be measured. A comparison between rates of flow on the top and bottom of a cladode could be done to observe if settling on the bottom of a cladode increases food accessibility to food through forces such as gravity.

A number of further studies can be using manipulation of light levels. By placing light on both sides of a plant when transferring crawlers to plants, it can be observed if light is a determining factor on where insects choose to settle. The same experiment can be performed in the absence of insects and then phloem extracted to see if light or the insects affect the plant's nutritional levels.

Conclusion

The difference in size of female cochineal insects settling on sheltered surfaces compared to those settling on exposed surfaces cannot be adequately explained using results obtained in this study. Varying levels of polyphenols are also unable to account for the trend in variation of insect size. This could be due to the small sample size available for analysis.

With further sampling amino acids may yet prove to be informative in the search for answers. There are still a number of opportunities to further the results found in these experiments and to expand on the knowledge obtained about biocontrol agents and the intricate relationships they have with their host plants.

Acknowledgements

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Table2. Comparative analysis of amino acids found in the phloem of two cladodes

Key: (D) = Detached
(P) = Potted
exp = exposed
shelt = sheltered

		cladode (D) exp		**Cladode (D) shelt**		**Cladode (P) exp**		**Cladode (P) shelt**	
Component name		**Raw amount (nmol)**	**% Total**	**Raw amount (nmol)**	**% Total**	**Raw amount (nmol)**	**% Total**	**Raw amount (nmol)**	**% Total**
Aspartic acid		7.67	6.26	3.06	3.87	5.17	5.37	11.53	6.30
Threonine		8.99	7.33	5.12	6.48	6.74	7.00	13.99	7.65
Serine		12.56	10.24	7.88	9.97	12	12.46	24.19	13.23
Glutamic acid		8.46	6.90	6.44	8.15	5.75	5.97	12.88	7.04
Glycine		8.52	6.95	5.72	7.24	6.95	7.22	11.98	6.55
Alonine		1.59	1.30	1.04	1.32	1.23	1.28	2.99	1.63
Valine		12.45	10.15	6.39	8.08	9.33	9.69	18.28	10.00
Methionine		2.99	2.44	1.53	1.94	2.43	2.52	3.9	2.13
Isoleucine		8.29	6.76	5.7	7.21	6.87	7.13	13.26	7.25
Leucine		9.33	7.61	5.62	7.11	8.13	8.44	17.56	9.60
Tyrosine		5.33	4.35	3.64	4.60	5.87	6.10	7.08	3.87
Phenylalanine		9.93	8.10	9.06	11.46	6.89	7.16	12.61	6.90
Histidine		7.43	6.06	3.61	4.57	4.88	5.07	7.98	4.36
Lysine		14	11.42	9.11	11.52	9.95	10.33	17.36	9.49
NH3		3.47	2.83	3.85	4.87	3.22	3.34	4.81	2.63
Serine		1.58	1.29	1.29	1.63	0.87	0.90	2.47	1.35
Total		**122.61**		**79.06**		**96.29**		**182.88**	

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