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Substrates and fertilization for the rustic cultivation of *in vitro* propagated native orchids in Soconusco, Chiapas

Anne Damon*, Marco Pérez Soriano, and Maria del Lourdes Rivera

El Colegio de la Frontera Sur (ECOSUR), Carretera Aeropuerto Antiguo, km. 2.5, Apdo. Postal 36, Tapachula, Chiapas, C.P. 30700, México.
*Corresponding author: adamon@tap-ecosur.edu.mx

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Abstract

Native orchid cultivation is a compatible alternative for impoverished coffee farmers in southeastern Mexico who are in crisis due to falling prices. Sustainable orchid cultivation is also an urgent necessity as an alternative to nonsustainable extraction from protected reserves, forest fragments and traditional coffee plantations, and to restore and conserve populations within these habitats. Our objective was to test the effects of locally available substrates and fertilizers upon orchids cultivated under typical rural conditions in coffee-producing areas in Soconusco, Chiapas. Seven species of epiphytic orchids native to Soconusco region—*Cattleya aurantiaca*, *Brassavola nodosa*, *Prosthechea* (*Encyclia*) *chacaoensis, Anathallis* (*Pleurothallis*) *racemiflora*, *Cattleya skinneri*, *Cycnoches ventricosum* and *Encyclia cordigera*—were propagated *in vitro*, acclimatized and established in rustic orchid galleries in the home gardens and plantations of coffee growers. Locally available waste products were used as substrates: clay tiles, tree bark, bamboo, seed hulls of pataxte (*Theobroma bicolor*) and wire baskets filled with bark chips. Two cheap and readily available commercial foliar feeds, Algaenzims (an organic product) and Bayfolan (a synthetic product) were tested. First, the substrates alone were tested for a period of 6 months to 1 year, then a combination of substrates and fertilizers were tested for 6 months, for effects upon leaf and root growth and root number. The mortality rates of these nonsymbiotically propagated, epiphytic orchids during the acclimatization phase, prior to these experiments, were high, between 60 and 90%. Once established in rustic galleries, the young orchid plants showed no preference for a particular substrate, survival depended upon technical problems during establishment, relating to difficulties with the attachment of plants to substrates, and the variable quality of care and attention offered by the farmers. Both fertilizers significantly improved one or all the parameters studied, and possibly counteracted the negative effects of the absence of symbiotic fungi, which, under natural conditions, are essential for orchid seed germination and adequate development of the young plant. More than half of the producers did not continue with the orchid cultures for economic and cultural reasons.

Key words: orchid cultivation, epiphyte, *in vitro* propagation, substrate, clay tiles, bark, bamboo, fertilization, acclimatization, conservation, coffee

Introduction

In this paper we present a general introduction to a relatively unknown sustainable option for coffee growers and, specifically, a study of the response of acclimatized, *in vitro* propagated plantlets of native, epiphytic orchids, to various locally available substrates and two brands of foliar fertilizer, within the context of the cultural conditions offered in the rustic galleries set up in the garden plots of coffee producers in six communities in the region of Soconusco, Chiapas. Epiphytic and terrestrial orchids are increasingly scarce in both natural and agro-ecosystems throughout the world, due to nonsustainable exploitation, and the deterioration, fragmentation or destruction of their habitats[1]. Epiphytic orchids are effectively mechanical parasites, they require other plants as supports but do not extract nourishment from their support plants. However, Benzing[2], cited by Koopowitz[3], suggested that under natural conditions they may be considered as nutritional pirates, competing with host trees for scarce and rapidly recycled nutrients. These plants require groups of mature trees, with trunks and branches covered in a rich flora of lichens, mosses, algae and fungi, which also implies the presence of a variety of cohabiting microorganisms, as well as insects, reptiles, amphibians and birds. That quality of substrate, found in tropical and temperate forests, takes several years to develop[4], during which epiphytes may be absent until the later stages of succession. Migenis and Ackerman[5] studied 11 species of orchids in a subtropical humid forest in Puerto Rico and emphasized the importance of the size (i.e., age) of the tree as a determinant factor for orchid presence. To recover a deteriorated forest may take several decades, and epiphytes may never recolonize the trees if there are no nearby sources of seeds and mycorrhizal fungi to facilitate the germination of those seeds[6,7]. Orchids may be more frequent on certain tree species, as discussed by Benzing[2], but are usually not specific[8]. Preferences for substrates may relate more to the prevalent ecological conditions[9] and the distribution and preferences of the mycorrhizal fungi, than to the nature of the support itself.

Mexico is one of the biodiversity hotspots in the world, within which Chiapas is the second most biodiverse state, with neighboring Oaxaca at the head of the list. More than a thousand species of epiphytes have been described in the country as a whole, and many of these are currently designated as in danger of extinction and live precariously in forest fragments, coffee plantations and solitary, often senile trees in pastures.

The region of Soconusco in the state of Chiapas in southeastern Mexico comprises the coastal lowlands and Pacific slopes of the Sierra Madre mountains that line the west coast of Mexico. It is a region renowned for its rich alluvial soils and agricultural production, but which is now suffering from a population explosion, immigration and emigration, contamination and advanced deforestation, as well as complicated political, social and economic problems. Agricultural production in Mexico in general is suffering competitive pressure from all sides, and coffee, one of the main agricultural products, has been particularly severely affected. Both Arabica (*Coffea arabica* L.) and Robusta (*C. canephora* Pierre ex Froehner) coffees are grown, on the higher and lower slopes of the Sierra Madre, respectively. Coffee can be grown using various systems, from the extremes of high-technology, high-input, shadeless coffee, to rustic, organic/biodynamic/ecological systems under shade trees, and with different certification requirements which may include such aspects as terracing on slopes, the provision of food sources for migratory birds, environmental education for the children of plantation laborers and the maintenance of shade tree diversity. The ideal altitude range for coffee-growing is 600–1500 m and this coincides roughly with the zone of maximum diversity of vascular epiphytes in the region, which was confirmed by Wolf[10] as 500–2000 m.

Within this scenario, therefore, coffee plantations are important for people and for many other organisms, but diversification of production is an urgent necessity for the economic and social well-being of producers. In the long term, the cultivation of native orchids could be a compatible, viable option for coffee producers, and the certified product can be sold in various forms (e.g., cards, invitations and wall-hangings with pressed flowers; conserved, three-dimensional flowers in presentation boxes; whole plants with flowers on a variety of supports; flasked *in vitro* plantlets where money is available for investment in equipment) and also used to repopulate plantations and depleted forests. Orchids can be collected from the ground, fallen branches, felled and rotten trees, or they can be propagated *in vitro*, by seed[11]. The use of tissue culture methods is not recommended here, not only because genetic diversity and plasticity are sacrificed[3], but also because of the costs involved. All species of orchids are protected internationally by CITES and within Mexico by the NOM-059-ECOL-2001[12], a situation that has been shown to be both corruptible and to deter many positive contributions to the conservation of these plants[3]. Furthermore, laws referring to the conservation of flora and fauna are barely recognized in most parts of the developing world, and few are willing to pay a suitably high price, that would reflect the scarcity and importance of the product and the challenges that represent attempts to cultivate it successfully. One of the most difficult aspects of this type of project is the awakening of interest in a product derived from protected species, which is usually followed by a surge of illegal collection and copycat, but uncertified, products offered at much lower prices.

As part of a multidisciplinary group effort set up by ECOSUR[13] to ameliorate the social, economic and environmental crisis in the coffee-growing areas of the southeast of Mexico, a pilot project for the rustic and sustainable production of orchids has been set up. One of the aims of this group is to offer alternatives so that impoverished coffee growers feel less inclined to erase or transform shaded coffee plantations, which effectively replace lost tropical forests and are vital as habitats for the persistence of many species of flora and fauna. The orchid project includes training, long-term assessment and plans for the certification of the product, both as an example to follow, and also to ensure that the product sells at an appropriate price. It is important to take into consideration the slow rate of growth and maturation for most species of orchids, which may take several years to produce their first flowers. There is virtually no literature concerning rustic, sustainable orchid cultivation, other than guides for amateur growers in developed countries, which would be inapplicable in rural Soconusco. Also, surprisingly, there is very little practical information concerning highly technical, commercial methods (*in vitro*, tissue culture, etc.), which often remain as 'in house' secrets of the major orchid-growing companies.

It would be considered logical to plant the orchids onto shade trees within the coffee plantations and harvest viable sections by division (vegetative propagation) at determined intervals; however, this is not possible, due to theft and competition between growers. In this program, the orchids

Figure 1. Orchid gallery constructed with wooden poles and 'manaca' palm leaves. The orchids, attached to substrates, hang from the wires that support the palm-leaf roof.

are grown in rustic structures called 'galleries' (Fig. 1), measuring from 4×4 m to 10×10 m, which are constructed within visible distance of the house, effectively forming part of the home garden or 'transpatio'. Posts can be made from wood but, for ecological reasons, concrete is preferable. Shade can be provided preferably by live trees or palm (usually 'manaca', *Scheelea preussii* Burret) leaves, but shade cloth can be employed where necessary.

Arboreal habitats and the epiphytes that inhabit them are complex and poorly understood. These habitats are usually considered as nutrient poor[14,15], giving rise to the need for obligate and facultative symbiotic interactions between coexisting organisms and epiphytes. In contrast, however, the analysis of the content of arboreal 'soils' (accumulated detritus, organic matter, canopy mats, etc.) in Costa Rican forests by Lesica and Antibus[16] revealed relatively high nutrient levels, and the latter authors mentioned other studies with similar results. Epiphytic orchids in all but the most humid environments feed in a dynamic and highly competitive environment for short periods only, during rainfall, and typically absorb nutrients dissolved in rainwater rapidly and efficiently, via both roots and leaves[17]. Orchids are also characterized by their ability to store water and nutrients in fleshy pseudobulbs and/or leaves, indicating permanent or temporal scarcity. Regular, low-dosage foliar fertilization can, therefore, be considered an appropriate method for the rustic cultivation of native orchids. Substrates for orchids can be natural, which may include nonrenewable resources such as tree-fern fiber (however, bear in mind the protected status of some species of tree ferns) and waste products, such as coconut fiber, which retain sufficient water[18], or artificial substances, which are often expensive and difficult to obtain. In cultures where orchid cultivation is a centuries-old tradition, materials used as fertilizer are diverse and often highly creative and idiosyncratic; Hew[19], reviewing ancient Chinese practices, mentions the use of goat and goose manure, and water in which fishes have been washed, as fertilizer.

Materials and Methods

The project began with the testing of four culture media for the propagation by seed, *in vitro*, of seven native species of orchids:

1. First phase (2000–1): *Cattleya aurantiaca* (Bateman ex Lindley) P.N. Don; *Brassavola nodosa* (L.) Lindley; *Prosthechea* (*Encyclia*) *chacaoensis* (Reichb.f. Dressler & Pollard). W.E. Higgins.
2. Second phase (2001–2): *Anathallis* (*Pleurothallis*) *racemiflora* (Sw.) Pridgeon & M.W. Chase; *Cattleya skinneri* Bateman; *Cycnoches ventricosum* Bateman; *Encyclia cordigera* Kunth Dressler.

The *in vitro* propagation was carried out as a service provided by the 'Centro Internacional de Investigación y Capacitación Agropecuario' (CIICA) in Ciudad Hidalgo in Chiapas, Mexico. The response of the seeds and plantlets of these orchids to the different culture media used was varied and protocols were established for *B. nodosa* and *C. aurantiaca*[20]. Plantlets were acclimatized for a period of 6 weeks in protected units in CIICA before being passed to the Regional Botanical Garden 'El Soconusco' in the municipality of Tuzantán, Chiapas, for further adaptation to field conditions. The process of acclimatization caused high mortality rates for all species, between 60 and 90%. The time from seed germination to the delivery of the surviving plants to the orchid galleries varied from 1 year to 18 months.

The acclimatized plantlets of the seven species of *in vitro* propagated orchids were delivered to the orchid galleries in rural coffee-producing communities in Soconusco, and fixed onto, or planted into, five substrates: bark, wire baskets filled with bark chips, clay tiles, bamboo and hulls of pataxte seeds, *Theobroma bicolor* Bonpl (a relative of cocoa, *T. cacao* L.). All plants, once attached to their substrates, were suspended by sections of cable from the wires that effectively form the 'roof' of the rustic galleries, at a height of approximately 1.80 m above the ground (Fig. 1). Plants were left to settle down for a month before data were taken, and mortality during this period was not taken into account. At first, responses to the different substrates alone were compared during a period of 6 or 12 months (second phase and first phase, respectively). This was followed by a comparison of the responses to two foliar fertilizers, 'Algaenzims' (BASF, New Jersey, USA) and 'Bayfolan' (Bayer Mexico, Mexico City) in combination with the different substrates, during a period of 6 months.

The materials chosen as substrates were all locally available and natural substances, except for the wire used for the baskets; and some could be considered as waste products, such as: clay roofing tiles, pataxte hulls, bamboo, bark and the bark chips used to fill the wire baskets. Bark from 'cedro' (*Cedrela odorata* L.: *Meliaceae*), 'roble' (*Tabebuia rosea* (Bertol.) A. DC.: *Bignoniaceae*) and 'primavera' (*Cybistax donnell-smithii (Rose) Seib.: Bignoniaceae*) were used and cut to various sizes (30×20 cm to

50×30 cm), depending upon the size of the orchid plants. Small plants without pseudobulbs were attached by small patches of superglue, and larger plants were tied on using 50-caliber nylon fishing line. Substrates were selected according to availability and producer choice, for that reason there were insufficient replicates to permit statistical analysis of the data for some of the substrates. The fertilizers selected were chosen because of their price, availability and the fact that they represent different types and formulations of fertilizer.

Algaenzims (BASF) is an organic product, with 4% of the organic matter comprising seaweed. It contains a wide range of minerals and trace elements which may be limiting factors in epiphytic habitats, and small quantities of plant hormones (cytokinins and gibberellins) which variously affect growth and root, bud and shoot development, depending upon concentration, the timing of application and combinations with other hormones[21].

Bayfolan (Bayer) is a synthetic product which, according to product information, comprises: 11% N (derived from ammonium nitrate, potassium nitrate, biammonium phosphate and ammonium sulfate), 8% P_2O_5 (biammonium sulfate and phosphoric acid) and 6% K_2O (potassium chloride, potassium nitrate and potassium sulfate). The vitamin B_1 content favors root growth, nutrient assimilation and respiration, whereas the presence of auxins (indoleacetic acid) promotes cell extension, tissue differentiation, respiration and shoot development. The product also contains chelated micro- and macroelements and a wetting agent.

In these experiments the results from the different galleries were pooled and, for the purpose of analysis, were considered as a single site, representative of the environmental conditions present in the coffee-growing areas of Soconusco. The process of the setting up of the orchid cultures and the subsequent care of the plants were standardized; all producers received the same training.

Reaction to substrate

Fifteen producers participated in this first stage of experimentation, comprising a total of 2963 plants (Table 1). After the plantlets were placed onto or into their substrates and suspended from the roof of the rustic galleries, monthly evaluations were carried out, which included discussions with the producers about any problems they might be experiencing, the amount of shade, incidence of pests and diseases, and the water requirements of the plants. Data were taken of the number of healthy, sickly and dead plants, for each orchid species within each substrate category, during a period of 12 months (first phase) or 6 months (second phase). At the end of the experimental

Table 1. Percentage survival of acclimatized plantlets, at 18 months (first phase) and 1 year (second phase), of orchids propagated *in vitro* by seed and placed in rustic galleries in the home gardens of coffee producers, in Soconusco, Chiapas. Percentages emphasized in bold and those underlined are the species with the highest survival rates, and the preferred substrates for each species, respectively.

Orchid species (total % survival)	Original number of plants	Substrate: Bark	Clay tiles	Bamboo	Wire basket and bark chips	Pataxte (small sample size)
First phase (at 18 months)		--------Percentage survival ± standard deviation across galleries-------- --------(number of galleries, total number of plants in trial)--------				
Cattleya aurantiaca (**67%**)	905	65 ± 29% (14, 314)	64 ± 26% (14, 289)	<u>70</u> ± 12% (9, 169)	65 ± 30% (14, 122)	<u>82</u> ± 17% (3, 11)
Brassavola nodosa (**67%**)	991	<u>67</u> ± 31% (14, 991)	63 ± 22% (13, 284)	61 ± 27% (10, 217)	61 ± 32% (14, 133)	<u>67</u> ± 30% (3, 12)
Prosthechea (*Encyclia*) *chacaoensis* (45.08%) (small sample size)	122	<u>52</u> ± 40% (13, 46)	<u>44</u> ± 43% (12, 55)	10 ± 23% (8, 21)	30 ± 28% (6, 10)	29 ± 29% (3, 7)
Second phase (at 1 year)						
Encyclia cordigera (**79%**)	360	<u>79</u> ± 18% (7, 132)	<u>80</u> ± 20% (7, 64)	<u>78</u> ± 25% (7, 91)	<u>80</u> ± 29% (5, 39)	<u>86</u> ± 23% (1, 4)
Anathallis (*Pleurothallis*) *racemiflora* (41%)	248	43 ± 19% (7, 104)	<u>64</u> ± 36% (6, 47)	29 ± 14% (7, 68)	–	24 ± 18% (4, 29)
Cattleya skinneri (56%)	216	65 ± 24% (7, 66)	29 ± 23% (5, 38)	62 ± 33% (6, 60)	<u>75</u> ± 23.1% (4, 16)	39 ± 45% (5, 31)
Cycnoches ventricosum (11.57%) (small sample size)	121	19 ± 30% (7, 54)	<u>41</u> ± 22% (5, 19)	3 ± 6% (5, 29)	<u>40</u> ± 12% (2, 5)	0 ± 0% (3, 13)

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Table 2. Distribution of orchid species and substrates.

Orchid species	Total number of plants	Substrate			
		Bark	Clay tiles	Bamboo	Wire basket and bark chips
Brassavola nodosa	279	144	101	17	17
Cattleya aurantiaca	215	94	88	15	18
Cattleya skinneri	18	18	–	–	–
Encyclia cordigera	103	56	15	15	17
Prosthechea (E.) chacaoensis	47	18	29	–	–

period, data were pooled and percentage survival of each species of orchid was calculated for each substrate.

During and at the end of the first phase, the producers who did not dedicate sufficient time and attention to their plants were eliminated from the project, and the second phase was carried out with the participation of the six most committed producers. The surviving plants from phase 1 were used for the trials in phase 2, but there were too few plants of *C. ventricosum* and *A. (P.) racemiflora* for inclusion in the second phase.

Reaction to fertilizer plus substrate

Six producers participated in this second stage of experimentation, all having proved to be interested in the project and consistent in their care of the young orchid plants. The substrate pataxte was not included at this stage due to insufficient material. A total of 662 plants were included in these trials (Table 2).

In each of the six rustic orchid galleries, for each orchid species and each substrate, the number of healthy plants available was divided equally and randomly among the three treatments: control; Algaenzims at half dose (as indicated for ornamental plants in the instruction leaflet); and Bayfolan, similarly at half dose. The plants were labeled with different colored tags, according to treatment, and were sprayed with one of the fertilizers or with chlorine-free, clean water, every 15 days during the growing (rainy) season for a period of 6 months, from April to October 2003. According to designation, the fertilizers or chlorine-free water were applied evenly to the plants by technical staff of the project, using the same sprayer for each treatment, or control, throughout.

At the beginning of the experiment, for each plant selected for inclusion in the study, measurements were taken of the length of the largest leaf and length of the longest root and the total number of roots. Measurements were repeated at 3 months and at the end of the 6-month experimental period.

Using three parameters chosen to define development (growth of longest leaf, number of roots, growth of longest root), comparisons were made between the development of the young orchid plants using four substrates (bark, clay tiles, wire basket, bamboo) and three treatments (control, Algaenzims, Bayfolan).

For statistical analysis, differences were calculated between the initial measurements and the measurements at 3 months (T1) and between the initial measurements and those at 6 months (T2). Data taken from the six galleries were pooled and analyzed using STATISTICA 6 (StatSoft, Tulsa, Oklahoma, USA) software. A univariate repeated measures analysis of variance (ANOVA) was applied and orthogonal contrasts were used to compare means.

Results

Reaction to substrate

The numbers of plants surviving to the end of the first phase was very variable, and related more to the quality of care offered by the producers than to the substrate. Furthermore, each producer used materials that were available to him, and the quality of such materials, such as bark and bamboo, was also variable. Data from this first phase were not suitable for statistical analysis, but percentage survival of the plantlets, in relation to each substrate, was calculated as a rough guide (Table 1). Survival was very similar for most substrates for both *E. cordigera* and *B. nodosa*.

More than half of the producers were eliminated from the project due to inadequate care of their plants. Once the plants were installed, many producers were unable to visualize the long-term benefit of caring for very small plants that grew very slowly and would flower in 4–6 years. During introductory talks, the realities of orchid cultivation and the importance of long-term and sustainable cultivation strategies were explained and discussed at great length prior to the installation of the crop, whereupon most producers declined to enter the project. Despite further training and long-term follow-up and assessment, a proportion of those who did commit themselves did not absorb the information and still hoped for a miracle crop that would solve their economic problems in a minimum of 6 months. Furthermore, displaced populations with no historical ties to their current plots, lack of general education, poor role models, environmental deterioration, lack of governmental commitment, corruption and mass emigration to the Unites States make it very difficult for new and long-term visions to be adopted by rural campesinos; they do not believe in themselves, their land or their government, and are more accustomed to

Table 3. Leaf and root growth of *Brassalova nodosa*, at time 1 (T1) and time 2 (T2), as influenced by substrate type and fertilizer treatment.

	Substrate					
	Clay				Bark	
	Leaf growth (cm)		Root growth (cm)		Leaf growth (cm)	
Treatment	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**
Control	0.35a	0.74a	0.31a	0.60a	0.41a	0.84a
Algaenzims	0.56b	1.28b	0.52b	1.04b	0.63b	1.25b
Bayfolan	0.67b	1.31b	0.56b	1.17b	0.67b	1.28b

Significant differences (at $P = 0.05$) within a column are indicated by different letters.

short-term handouts than to well-planned, hard work. Within that context we consider it a victory to have maintained these producers within the project for several years (some are now in their fourth year).

Reaction to fertilizer plus substrate

For various reasons, as explained in the discussion, survival was poor and there were insufficient data for statistical analysis for *A*. (*P*.) *racemiflora* and *C. ventricosum*, and very few data for *P*. (*E*.) *chacaoensis*. Statistical analysis could not be carried out for number of roots, due to the large number of zeros in the data; roots proved to be attractive to foraging small birds, insects and reptiles, especially basilisks (*Basiliscus* sp. Squamata: Corytophanidae).

Statistical analysis was carried out for bark and clay tiles for the three orchid species that showed highest survival rates. Some plants were lost and the numbers of plants that survived to the end were fewer than those presented in Table 2. There were no cases of a significant difference between the two fertilizers.

***Brassavola nodosa*.** Plants on clay tiles showed significantly better leaf and root growth with both fertilizers, as compared to the control, and plants on bark showed significantly better leaf growth with both fertilizers (Table 3).

***Cattleya aurantiaca*.** Plants on clay tiles showed significantly better leaf growth with both fertilizers, as compared to the control, and plants on bark showed significant improvements in leaf and root growth (Table 4).

***Encyclia cordigera*.** For plants planted onto bark, Algaenzims and Bayfolan promoted significantly better leaf growth as compared to the control (Table 5).

Where statistical analysis was not carried out due to low numbers of repetitions, numerical advantages were observed in most cases for one or both fertilizers, as compared to the control, and were particularly notable for the following: bamboo, *E. cordigera*, leaf growth with Algaenzims; wire basket with bark chips, *E. cordigera*, leaf growth with Algaenzims; bark, *C. skinneri*, number of roots with both fertilizers; clay tiles, *P*. (*E*.) *chacaoensis*, leaf and root growth, with both fertilizers.

P. (*E*.) *chacaoensis* gave a poor response to all the substrates offered and was susceptible to infection by the fungus *Myrothcium* sp. (identified by Carlos Garibay-Gálvez, CIICA, 2001) at all stages of the study, from *in vitro* germination of seeds, through acclimatization, to growth and development in the rustic galleries, despite being the most common, widely distributed, resistant and weedy species of orchid found in Soconusco region.

A. (*P*.) *racemiflora* responded relatively well at all stages, and most losses were due to the technical problems

Table 4. Leaf and root growth of *Cattleya aurantiaca*, at time 1 (T1) and time 2 (T2), as influenced by substrate type and fertilizer treatment.

	Substrate					
	Clay		Bark			
	Leaf growth (cm)		Leaf growth (cm)		Root growth (cm)	
Treatment	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**
Control	0.40a	0.88a	0.41a	0.81a	0.37a	0.70a
Algaenzims	0.61b	1.11b	0.66b	1.24b	0.49b	0.95b
Bayfolan	0.73b	1.38b	0.63b	1.24b	0.63b	1.21b

Significant differences (at $P = 0.05$) within a column are indicated by different letters.

Table 5. Leaf growth of *Encyclia cordigera* at time 1 (T1) and time 2 (T2), as influenced by substrate type and fertilizer treatment.

	Substrate	
	Bark	
	Leaf growth (cm)	
Treatment	**T1**	**T2**
Control	0.32a	0.76a
Algaenzims	0.62b	1.22b
Bayfolan	0.49b	1.11b

Significant differences (at $P = 0.05$) within a column are indicated by different letters.

associated with fixing the plantlets onto their substrates; this species lacks pseudobulbs and the plantlets were too small to be tied onto the substrates. After elimination of other methods for various reasons, a brand of 'superglue' was used which proved nondamaging to the plants. The technique functioned best with clay tiles; however, many of the plants became unstuck and fell to the ground, where they disappeared from sight. This small plant did not adapt well, at this stage, to a horizontal existence in the wire baskets, but larger plants have been transplanted successfully into baskets.

C. skinneri was delicate at all stages of production and, like *P.* (*E.*) *chacaoensis*, was susceptible to fungal infections. The response of this showy species was similar for wire baskets, bark and bamboo, but mortality was higher for clay tiles and pataxte.

C. ventricosum is a beautiful, deciduous orchid which remains leafless throughout the 6 month dry season that is typical of the Soconusco region. The *in vitro* propagation of this species was difficult and presented various novelties. At one stage the plants shed their leaves and the pseudobulbs suddenly turned a dark brown color and were about to be thrown out, when a flush of new leaves was produced throughout the culture. The plantlets produced round pseudobulbs early on in their development and lost and replaced their leaves every 2 or 3 months, which was probably an exaggerated deciduous response to the cultural conditions, as compared with wild, mature plants which drop their leaves once, at the beginning of the dry season, to be replaced 6 months later at the onset of the rains. The plants of this species were also difficult to fix to the substrates and many were lost due to problems with the glue, as for *A.* (*P.*) *racemiflora*.

By comparing the controls, the results suggest that bark offers better conditions than clay tiles for *B. nodosa*, but that bark and clay tiles offer very similar conditions for *C. aurantiaca*. It is interesting to note that the fertilizers promoted significantly better leaf and root growth for most plants on clay tiles but only improved leaf growth for plants of *C. aurantiaca* on bark, suggesting that bark supplies sufficient nutrients for root growth, but not for leaves.

Discussion

In the first phase of this study, the nature of the substrate itself had little effect upon the survival rate of plantlets of the most robust orchids, *E. cordigera*, *B. nodosa* and *C. aurantiaca*, in that order. In the second phase, reactions between substrates and orchid species were variable; there was, however, a clear trend that both fertilizers improved the development of the orchids in at least one of the parameters studied.

Orchid plantlets produced asymbiotically *in vitro* are notoriously difficult to adapt to natural conditions; the epidermis of plants contained within the sealed, sterile flasks is very thin and plants are thus vulnerable to pests and pathogens and dehydrate very quickly. The plants require slow, progressive changes in order to acclimatize to fluctuations of humidity and temperature and invasion by bacteria and fungi[22]. For all seven species of orchids in this study, the numbers of surviving plants 3 years after germination *in vitro* was extremely low, which can be attributed to the variable quality of attention given to the plants by the farmers and the lack of a suitable mycorrhizal fungal inoculant, which under natural conditions facilitates the nutrition and thus fortifies young plants; in these experiments the plants were all of unselected, wild origin.

Under natural conditions, orchids feed upon sources of N, P and K dissolved in rainwater and organic matter trapped between forked branches, and in fissures and rough surfaces of tree bark, as well as amongst the roots, leaves and stems of climbers and epiphytes. Rainwater is obviously varied in its composition, but, as a reference, the analysis done by Ospina[23], in the Central Amazon, indicated that water containing $2.11\ mg\,l^{-1}$ Na, $0.1\ mg\,l^{-1}$ K, $0.07\ mg\,l^{-1}$ Ca, $0.02\ mg\,l^{-1}$ Mg and $0.095\ mg\,l^{-1}$ P was the main nutrient source of orchids. However, the study did not include analysis of leachates from leaf litter and direct deposition of invertebrate (and sometimes vertebrate) carcasses and excrement.

The requirement for fertilizers depends upon the nature of the substrate. According to Arditti[17], most organic materials that undergo moderate degradation do not require fertilizer, but tree bark has been shown to be an exception and additional fertilization is recommended. The ability of bark to retain water varies according to age[3] and species.

The benefit gained by the application of fertilizers reflects, in part, the capacity of the substrate to saturate the requirement for minerals and N, P, K of the young orchid plants. Bark is mentioned in the literature as a nutrient-deficient substrate, and numerical or significant improvements were observed with both fertilizers in most cases, but mainly for leaf growth, the growth of the longest root was not much improved. The quality of bark was very variable between the different galleries, with variation due to different species of tree, different ages of the living tree (which is reflected by the microflora present) and the state of decomposition of the bark. Wire baskets filled with pieces of bark also showed a general improvement with both fertilizers, for all three parameters, but to a lesser degree. In this case, the accumulation of organic detritus due to the activities of ants and numerous other insects cohabiting between the layers of bark probably contribute to orchid nutrition. Wire baskets are not, however, advisable in very humid and shady environments, due to the accumulation of excessive humidity which causes rotting of the plants, worsened if the farmer insists on watering regularly without checking the prior condition of the plant and substrate. Clay, being compressed, baked earth, should be a source of various minerals and N, P and K; however, in drier and sunnier areas this substrate requires more frequent watering and, without moisture, the mineral content becomes inaccessible. This substrate was shown to be equal, or very slightly inferior, to bark as a substrate in this study, probably due to lack of watering. Bamboo benefited from the application of both fertilizers, and Algaenzims was especially efficient at promoting leaf growth for this substrate. Neither fertilizer showed clear superiority for promoting leaf or root extension in any of the substrates.

Conclusions

This century, product diversification on a wide scale will be essential to combat poverty, market domination and environmental degradation. The production of native orchids is a promising alternative for coffee farmers in the Soconusco region and may be combined with ecological and organic coffee, ecotourism and the elaboration of arts and crafts. Similarly, epiphytic bromeliads are another alternative, and Wolf[24] suggests methods for evaluating populations and determining sustainable extraction quotas.

Many campesinos in the Soconusco region of Chiapas are reluctant to commit themselves to programs promoting diversification, secondary economic activities and new solutions to old economic problems, and even less so if these programs imply waiting for long-term results. Such programs are tainted with a history of failure, and most options are presented to producers via poorly planned and corrupt programs linked to ephemeral political interests. In short, producers lack good role models; they have no evidence that new techniques, dedication and attention to detail reap benefits. Many of the producers involved in this program participated only half-heartedly and were incapable or unwilling to learn and apply the techniques offered. None the less, a proportion of the coffee farmers who joined this program for the rustic and sustainable cultivation of orchids achieved positive results, which could be traced back to an enthusiasm for learning and mastering a new crop, attention to detail, regular revisions of the young plants and personal initiative when rapid solutions were necessary for unforeseen problems. We feel that key points for the success of this type of project are an adequate selection process prior to commitment to the project and frequent and long-term assessment.

Orchids grow very slowly, and the plants in this study, at 3 and 4 years old, were still very small. The significant gains in length of leaves or roots, or the number of roots, although perhaps not fully appreciated by the orchid growers, were nevertheless highly significant for both plant and grower. Pests and diseases were not a significant problem, but one pest, a mirid bug[25], when it occasionally appeared, required very prompt action to avoid damage.

A range of organic waste products can be used for rustic orchid cultivation without affecting the establishment and development of the orchid plants, provided that basic requirements for water and shade are met. Low-dosage foliar fertilization is an appropriate method for the rustic cultivation of native orchids. Algaenzims and Bayfolan, applied at half dose, in combination with clay tiles, bark, wire baskets filled with bark chips and bamboo substrates significantly increased leaf growth and/or root growth of acclimatized, 3- and 4-year-old plants of five orchid species propagated *in vitro* and established in rustic galleries in rural communities in the Soconusco region.

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