VARIATIONS IN THE QUASSIN AND NEOQUASSIN CONTENT IN Quassia amara (SIMAROUBACEAE) IN COSTA RICA: ECOLOGICAL AND MANAGEMENT IMPLICATIONS

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Abstract

Because of the quassinoids present in its tissues, the tropical shrub, Quassia amara (Simaroubaceae) is used to make medicinal extracts and insecticides. By means of high performance liquid chromatography, the levels of quassins and neoquassins were measured (0.28%, 0.20%, 0.16%, and 0.14% in branches with diameters of > 4.5 cm, 3.0 - 4.5 cm, 1.5 - 3.0 cm, < 1.5 cm respectively). Regression models were used to evaluate biomass and quassinoid quantities at 4 pruning heights (0, 30, 50 and 100 cm) considering the shrubs' height and diameter (30 and 10 cm from the ground). It was found to be better to cut thick shafts and to prune low.

The quassin content was not significantly different in populations from 5 climatic regions in Costa Rica, but the neoquassin content did vary between 0.09% and 0.17%, and was higher in individuals found in very humid forests. The hypothesis proposed here is that quassinoids accumulate in the shrub's xylem as it grows, and that more chemicals are produced under less favourable growing conditions, such as those found in humid and very humid forests where the available light is limited.

The distribution study of the species in Costa Rica shows denser populations in areas with less than 450 m above sea level with unlimited water supply, good drainage and high light levels. In areas with less than 2500 mm of annual precipitation, the shrub is found only in riparian forests. In humid and very humid forests (with up to 5500 mm of precipitation) the shrub is more prevalent in areas with higher light levels. A plan for the sustainable use of the species in a natural population in Costa Rica is discussed.

1. Introduction

Quassia amara is a shrub found from the 18° latitude in Mexico to Ecuador and Brazil where its medicinal use in the treatment of fevers, digestive complications and intestinal parasites is well known. Although it has historically been used mainly for medicinal purposes, extracts from the plant have been widely used in the soft drink industry and to make natural insecticides in Europe, the United States. The woody plants are imported from South America and the Caribbean (Busbey 1939, Brown 1995, Holdridge and Poveda 1975).

In 1994 a drug company offered to buy 500 kg of Q. amara per month from the Kekoldi Indigenous Reserve on the south Atlantic coast of Costa Rica, where there is a natural population of the shrub which covers approximately 100 hectares. At that time, the methodology proposed by Marmillod and Villalobos (1997) to determine the natural populations of the resource, the possibilities for its sustainable harvesting and a silvicultural system was used.
Brown (1995) and Villalobos (1995) did not find diameters greater than 13 cm - measured at 0.3 m in height, \( d_{0.3} \) - in diverse natural populations in Costa Rica and Panama, nor did they find heights greater than 9 m. For the Kekoldi \( Q. amara \) population, Ling (1995) calculated \( d_{0.3} \) means from 2.18 cm and maximums of \( d_{1.3} \) from 6.16 cm and 6.5 m in height.

This article describes part of the process, developed by the Center for Tropical Agriculture Research and Higher Learning (CATIE) to define a sustainable use criteria for the natural populations of \( Q. amara \) which considers the product that needs to be obtained in the field in order to satisfy industry requirements.

2. Materials and Methods

2.1. Species Ecology

Quassinoids are documented as the basic active components in \( Q. amara \). Quassins and neoquassins are the main quassinoids present in the plant's tissues. The shrub's main products are its woody tissues. To determine the extent to which different types of branches produced quassinoids, 30 individuals which represented the full range of the species in Costa Rica, were studied in natural populations. The chemical analysis was repeated for 15 shrubs in five different natural populations.

The branches were separated into categories: fine (diameter <1.5 cm), thin (1.5-3.0 cm), medium (3.0-4.5 cm), and thick (>4.5 cm). They were cut at four different pruning heights (0.30, 50 and 100 cm above the ground). The shafts were weighed and samples taken from them were air dried for 72 hours at 50°C to determine their dry weight and chemical composition.

Dried, 6 gram xylem samples folded in filter paper (Whatman 41) were submerged in a Sohxlet extraction with ethanol (95%) for two and a half hours. The extracts were evaporated dry and re-dissolved in a 9:1 mixture of ethyl acetate and methanol. They were then filtered through Whatman 1 PS paper, evaporated, dried and re-dissolved in methanol in order to inject them into the HPLC to read the quassin and neoquassin I and II compound levels. Using the Fournival Index (1961), 15 possible regression models were evaluated to estimate the total fresh and dry biomass, as well as the quantity of quassinoids in function of the \( d_{0.1} \) and \( d_{0.3} \) (recommended by Steward et al. 1992) and the height of the shrub.

To determine the factors that control \( Q. amara \) distribution in Costa Rica, literature and herbarium data were analysed. The tendencies found were verified in the field and by checking against characteristics identified in cross-section measurements of 8 natural populations representative of the climates present within the distribution limits.

In cross-sections, of at least 1 km in length, 300 individuals were sampled which ran across each population and one or two of the environmental gradients of interest, including circular plots whose area (10 m², 50m² or 100m²) and distance (8m, 12m or 25 m), varied according to the density at the sampling point. In each plot, the following data were taken: altitude, slope, vegetation composition, topography, canopy height and light levels (Clark and Clark 1992) as well as the basal diameter (\( d_{0.3} \) in cm), height (h in cm), basal area (Gim m²), density (N/100m²) and dry biomass (kg/100m²).

2.2. Development of a Management Plan

Brown (1995) suggests pruning heights greater than 50 cm in order to take advantage of the species' sprouting capability. In Kekoldi, 90% of the biomass greater than 50 cm has branches with diameters over 2.5 cm (Marmillod et al. 1995); they are, therefore,
harvestable (B+0.5). A formula was developed to estimate the Kekoldi biomass in function of d0.3. Fifty-one individuals representative of the diametric dimensions present in the population were sampled.

An inventory was done on the main patch of Q. amara, which had been delimited previously with the help of local guides. Through sampling points consisting of plot pairs of 100 m² separated by distances of 100 x 25 m, 6.8% of the 100 hectare area was covered. Within these all, the individuals with ḍ>0.5 m were counted for usable stems (2 cm diameter with 1 m height). Data were taken on environment, canopy height and topography.

3. Results

3.1. Species Ecology

The xylem was found to have 40% moisture regardless of the thickness of the branch (p<0.05), but the bark had between 64.5% moisture in the fine branches and 58.1% in the thick ones. In addition, the proportion of the bark in the total fresh woody biomass is only 33.4% for fine branches, 24.9% in thin branches and 19.9% in the medium and thick ones. So the xylem is the main component in the dry harvested woody biomass.

It was found that when d0.1 was used as the basal diameter, the best possible model to estimate biomass or harvested quassinoids should include height measurements, while with the use of the ε0.3 it is possible to obtain good estimates without using this additional variable.

The quassinoid content was higher in direct proportion with the thickness of the branch (varying from 0.28%, 0.20%, 0.16%, to 0.14%, thick to thin branches respectively). The quassin content was 0.17%, 0.14% 0.10% and 0.09% respectively and the neoquassin content was 0.11%, 0.07%, 0.06% and 0.05% respectively.

The quassinoid content varied among natural populations with a tendency toward lower levels in less rainy areas that have higher light exposure (0.17% in thin branches in Kekoldi in the humid forest of the Atlantic and 0.09% in the Pacific in the dry forest). This variation was mainly a product of the variation in the content of neoquassin as there was little difference in quassin content between the populations.

Forty-four percent of the documented sites with Q. amara belong to the very humid tropical forest life zone according to the Holdridge classification system (1987), 26% is classified as very humid premontane in transition to basal, 11% was humid tropical forest and the rest was distributed in other zones all corresponding to basal floors or their transitions (Villalobos 1995).

The regions that receive the highest measured precipitation in Costa Rica are the flood plains of the extreme north Atlantic coast, where there is no dry season, and the extreme south Pacific zone. In the latter, there is lower total precipitation, there is a dry season and the terrain is more broken (Villalobos 1995, Barrantes et al. 1985, Herrera 1985, Rojas 1985).

The shrub is found on the north Pacific slope where the dry season is more marked and the landscape has a combination of great savannas and high mountain ranges. In this area the groups of Q. amara are sometimes separated by several kilometres. They are found exclusively in riparian forests around rivers and streams, although they are more extensive and dense than those seen in humid forests (Villalobos 1995, Barrantes et al. 1985, Herrera 1985, Rojas 1985).

Due to increasing precipitation and decreasing light exposure moving from the north Pacific plains to the south Pacific, the species is found in progressively smaller and more isolated groups. In secondary forests, the populations are composed of a few large
individuals and many small shrubs, while in dense primary forests, large individuals are common. Kekoldi, in the south Atlantic, has intermediate precipitation levels (2500-4000 mm annually) well distributed throughout the year. Here, the species is practically absent from the valley floors and it grows mainly on ridges with good drainage (Villalobos 1995, Ling 1995).

The densiest plots were observed in areas with the highest sun exposure (up to 14000 individuals/hectare). In areas with higher precipitation and greater cloud cover, the highest densities were between 600 and 700 ind/hectare, although a small population of 1800 ind/hectare was found in the south Pacific in an alluvial plain. This indicates that the species grows better under more fertile conditions than those found in the hills, when no other limiting factors are present.

Although the basal area values (G) came out higher in the north and central Pacific zones, the magnitude of that difference does not correspond to the density. The groups in these zones are composed of many small individuals, that is to say they are juvenile stands. The least altered sites have lower densities but larger, older individuals. Similarly, in the rainy sectors studied there are higher $d_{0.3}$ means in the topographically lower and less exposed plots (Ling 1995, Villalobos 1995). This is probably due to the higher regenerative activity in the more exposed areas.

3.2. Development of a Management Plan

The most practical model with best results to estimate usable biomass in the natural Kekoldi population ($B_{0.5}$), testing for individuals with diameters exceeding 2 cm was:

$$B_{0.5} = 0.238107 - 0.433307 \times d_{0.3} + 0.222039 \times d_{0.3} <$$

where $d_{0.3}$ is in cm h is in m and $B_{0.5}$ is in kg.

Based on the inventory of Q. amara in Kekoldi, the management areas are defined as: productive in use (where there have been previous harvests), productive usable (with individuals greater than $B_{0.5} = 2.5$ kg/100 m$^2$), productive but not usable, and non-productive (where none of the species is found).

The productive in use area, covered by deforested areas in different stages of advanced secondary succession and home gardens favourable for the growth and regeneration of the species, constitutes 3% of the managed area with close to 1500 kg/ha and 20% of the available $B_{0.5}$. Here the individuals with $d_{0.3}$ greater than 5 cm represent 66% of the remaining $B_{0.5}$. Considering an error of 41.4% the $B_{0.5}$/ha is greater than 890.4 kg/ha with 80% certainty. If the individuals with $d_{0.3} \geq 6$ cm (57 ind/ha) are left to seed, the $B_{0.5}$ the remainder is greater than 621.3 kg/ha.

The usable productive area (21% of the area) contains 80% of the available $B_{0.5}$. Two thirds are covered in virgin forest, 25% are dense thicket and the rest are secondary forest. The individuals are larger but have fewer branches, which indicates less favourable conditions for regeneration, sprouting and growth. There are 18 ind/ha with $d_{0.3} \geq 7$ cm but very few with $d_{0.3} < 1$ cm. The opening in the canopy in the first case could allow for the formation of regenerative patches. With an estimation error of 15%, $B_{0.5} > 703.7$ kg/ha with 80% certainty. Leaving the individuals with $d_{0.3} \geq 6$ cm (44 ind/ha) to seed there is a $B_{0.5} > 386.1$ kg/ha.

From a measurement taken in 8 Q. amara plantations in diverse environments in Talamanca, with plants of a known age, a mean increase in $B_{0.5}$ of 80 g/stem/year was estimated. The two usable zones produce 1887 kg/year. Given that the total $B_{0.5}$ is 12 tons, the first rotation was planned for six years from now with the possibility of annual cuts of 1800 kg thereafter.

Management in altered forest and thickets, which encompass 84% of the usable zones, was found to be essential. For altered forest it was recommended: that 60 plants/ha be
designated as seed plants; that over the period of at least 5 years, new patches have to be created for regeneration (dispersing seeds in natural clearings in the forests); that the growth of individuals in the existing patches should be promoted by opening apertures in the canopy; and that all the usable biomass in the cutting area should be extracted protecting the seed plants and thus eliminating the direct competition of useless plants or trees.

In the thickets, where the light exposure and *Q. amara* densities are higher, it is recommended that all the usable biomass shall be extracted except 60 saplings/ha and that the direct competition of useless species should be controlled. The goal is to create a less dense forest with a higher canopy composed of valuable timber species and a shrub stratum with dense patches of *Q. amara*. In both zones shoots are thinned the second year, leaving only the strongest.

4. Discussion

The *Q. amara* populations which contained a lower quassinoids content and grow in less rainy areas correspond to microenvironments that do not have important water limitations through the year, such as the riparian forests, where species regeneration, density and growth are greater than in the less altered rain forests, where the light availability is a limitation.

Apparently, the quassinoids are substances used for defence and are produced under unfavourable growth conditions. They accumulate in the plant over time. This is a common strategy among understory species in tropical forests (Bryant *et al.* 1983). It is probable that these tetracyclic triterpenes derivatives are produced in the leaves and transported via the phloem to the xylem where they accumulate in the older tissues in the thickest and lowest branches.

To date, the commercialization of the shrub has been based on the total weight of the branches harvested, without considering their moisture, diameter, length nor state of cleanliness. In a future market where the quality of the product, determined by its quassinoid content, will form part of the buying criteria, the production of thick branches will be of upmost importance.

The absence of the species in the montane forests and low montane forests that cover a great deal of the country indicate that altitude in this latitude is related to the temperature. This is a primary limitation to the distribution of *Q. amara* whose presence has not been documented above 500 m above sea level in Costa Rica (Villalobos 1995, 1996).

*Q. amara* is present in almost the whole range of precipitation conditions in Costa Rica’s lowlands according to the Holdridge (1987) and Herrera (1985) classification systems. The exceptions are very rainy areas (with more than 5500 mm of annual precipitation) which indicates limitations to the distribution of the shrub for reasons of drainage, cloud cover and other related factors. The availability of water determines the presence of the species below 500 m above sea level, where it appears to need a minimum level of moisture in the soil year-round without which it could not develop. While areas with abundant precipitation need well drained soils. Nevertheless, well-drained soils seem to coincide with higher availability of light; this interaction requires further study.

Sunlight averages cause an inverse effect on the mean values of the precipitation in the study sites from where a hypothesis has emerged that higher levels of light in areas where the soil moisture is not a limitation can promote growth and regeneration of *Q. amara* (in accordance with Brown's results 1995) and facilitate the development of natural populations.
These observations suggest that the riparian forests in the driest areas in Costa Rica are ideal for the regeneration and growth of the species because of the combination of conditions they possess: unlimited water, no excess of moisture in the soil, high sunlight levels due to the climate in the zone, the limited expanse of forest and its floral composition.

In Kekoldi, in the south Atlantic, the only place on this coast where the species grows in wild conditions, the indigenous peoples' farming practice of opening clearings in the forests promotes the survival of *Quassia amara* and its exposure to light has favoured the formation of dense groupings (Ling, 1995). Human intervention and other events that affect light exposure to an individual is a third factor which determines the presence of *Quassia amara*.

In the lowland regions, *Quassia amara* is only found under favourable climate conditions. Within these populations, the mean density analysis along each cross-section showed groups in sites that had optimum light and moisture conditions throughout the year. That is to say that in humid forests, the groups form on ridges and slopes. The current analysis does not confirm that soil conditions have a direct effect on the presence of *Quassia amara* (Villalobos, 1995).

CATIE's experience with regard to *Quassia amara* shows that it is possible to develop silvicultural criteria following a process where the ecology of the species is studied in function of the product desired. It is important to complement diverse levels of study to reduce the risk of generalizing patterns of ecology, growth and of active principle production.

*Quassia amara*'s characteristics show a resource that can be used in diversified forest management systems, where timber harvesting and other silvicultural activities would benefit from having more than one product.

Follow-up activities that can guarantee the sustainable management of a plant resource must be technical and scientific, generating new knowledge from each silvicultural project with the goal of to improve management both in the field of biology, as well as in marketing and administration.

5. References


