

Review

Studies on biochemical and physiological aspects in relation to phyto-medicinal qualities and efficacy of the active ingredients during the handling, cultivation and harvesting of the medicinal plants

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Medicinal and aromatic plants are mostly perishable and take more care for the careful handling and improve processing for better qualities and efficacy of the active ingredients in post harvest processing. Therefore, taking into the care of the secondary plant products viz., mono- terpeins, steroids and alkaloids contents and its properties with quality and quantity, should be handled properly. The biochemical and physiological characters in handling the quality will be of utmost care, after the harvest. In this attention a proper care has been adopted through this review, in handling the post harvest techniques on the biochemical and physiological processing of the secondary plant products and its active bio-molecules of the specialized medicinal and aromatic plants.

Key words: Medicinal plant, post harvest, phyto-medicinal qualities, efficacy of the active ingredients, cultivation and conservation.

INTRODUCTION

The past decade has witnessed a tremendous resurgence in the interest and use of medicinal plant products, especially in North America. Surveys of plant medicinal usage by the American public have shown an increase from just about 3% of the population in 1991 to over 37% in 1998 (Brevoort, 1998). The North American market for sales of plant medicinal has climbed to about \$3 billion/year (Glaser, 1999). Once the domain of health-food and specialty stores, phyto-medicines have clearly re-emerged into the mainstream as evidenced by their availability for sale at a wide range of retail outlets, the extent of their advertisement in the popular media and the recent entrance of several major pharmaceutical companies into the business of producing phytomedicinal products (Brevoort, 1998; Glaser, 1999; Blumenthal et al., 2000). No doubt a major contributing factor to this great increase in phytomedicinal use in the United States has been the passing of federal legislation in 1994 (Dietary Supplement Health and Education Act or "DSHEA") that facilitated the production and marketing of phytomedicinal products (Brevoort, 1998).

The past decade has also witnessed intense interest in "nutraceuticals" (or "functional foods") in which phyto-chemical constituents can have long-term health

promoting or medicinal qualities. Although the distinction between medicinal plants and nutraceuticals can sometimes be vague, a primary characteristic of the latter is that nutraceuticals have a nutritional role in the diet and the benefits to health may arise from long-term use as foods (that is, chemoprevention) (Korver, 1998). In contrast, many medicinal plants exert specific medicinal actions without serving a nutritional role in the human diet and may be used in response to specific health problems over short- or long-term intervals.

For many of the medicinal plants of current interest, a primary focus of research to date has been in the areas of phytochemistry, pharmacognosy and horticulture. In the area of phytochemistry, medicinal plants have been characterized for their possible bioactive compounds (Bruneton, 1995), which have been separated and subjected to detailed structural analysis. Research in the pharmacognosy of medicinal plants has also involved assays of bio-activity, identification of potential modes of action and target sites for active phytomedicinal compounds. Horticultural research on medicinal plants has focused on developing the capacity for optimal growth in cultivation.

This has been especially pertinent as many medicinal

plants are still harvested in the wild and conditions for growth in cultivation have not been optimized. Wild harvesting of medicinal plants can be problematic in terms of biodiversity loss, potential variation in medicinal plant quality and occasionally, improper plant identification with potential tragic consequences.

From the perspective of plant physiology, extensive opportunities exist for basic research on medicinal plants and the study of their phytomedicinal chemical production (Schröder, 1997). This review presents a discussion on some fundamental aspects of phytomedicinal chemical production by plant cells with an overview of several medicinal plants that have received considerable use and attention over the past decade.

PHYTOMEDICINAL ACTIONS OFTEN RESULT FROM SECONDARY PLANT PRODUCTS INVOLVED IN PLANT ECOPHYSIOLOGY

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. That the medicinal actions of plants are unique to particular plant species or groups is consistent with this concept as the combinations of secondary products in a particular plant are often taxonomically distinct (Wink, 1999; Fornasiero, 198). This is in contrast to primary products, such as carbohydrates, lipids, proteins, heme, chlorophyll and nucleic acids, which are common to all plants and are involved in the primary metabolic processes of building and maintaining plant cells (Kaufman et al., 1999; Wink, 1999).

Although plant secondary products have historically been defined as chemicals that do not appear to have a vital biochemical role in the process of building and maintaining plant cells, recent research has shown a pivotal role of these chemicals in the ecophysiology of plants. Accordingly, secondary products have both a defensive role against herbivory, pathogen attack and inter-plant competition and an attractant role toward beneficial organisms such as pollinators or symbionts (Kaufman et al., 1999; Wink and Schimmer, 1999). Plant secondary products also have protective actions in relation to abiotic stresses such as those associated with changes in temperature, water status, light levels, UV exposure and mineral nutrients (Kaufman et al., 1999; He et al., 1997).

Furthermore, recent work has indicated potential roles of secondary products at the cellular level as plant growth regulators, modulators of gene expression and in signal transduction (Kaufman et al., 1999; Nahrstedt, 1997). Although secondary products can have a variety of functions in plants, it is likely that their ecological function may have some bearing on potential medicinal effects for humans. For example, secondary products involved in plant defense through cytotoxicity toward microbial pathogens could prove useful as antimicrobial medicines in humans, if not too toxic. Likewise, secondary products

involved in defense against herbivores through neurotoxin activity could have beneficial effects in humans (that is, as antidepressants, sedatives, muscle relaxants, or anesthetics) through their action on the central nervous system.

To promote the ecological survival of plants, structures of secondary products have evolved to interact with molecular targets affecting the cells, tissues and physiological functions in competing microorganisms, plants and animals (for discussion, see Wink and Schimmer, 1999). In this respect, some plant secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules, or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites (e.g. central nervous system, endocrine system, etc.) (Kaufman et al., 1999). As noted by Wink (1999), the development of structural similarity between plant secondary products and the endogenous substances of other organisms could be termed "evolutionary molecular modeling."

THE BENEFITS OF PHYTOMEDICINES OFTEN RESULT FROM SYNERGISTIC ACTIONS OF MULTIPLE ACTIVE CHEMICALS

In contrast to synthetic pharmaceuticals based upon single chemicals, many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process. As pointed out by Tyler (1999), this synergistic or additive pharmacological effect can be beneficial by eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body. In this respect, Kaufman et al. (1999) extensively documented how synergistic interactions underlie the effectiveness of a number of phytomedicines.

This theme of multiple chemicals acting in an additive or synergistic manner likely has its origin in the functional role of secondary products in promoting plant survival. For example, in the role of secondary products as defense chemicals, a mixture of chemicals having additive or synergistic effects at multiple target sites would not only ensure effectiveness against a wide range of herbivores or pathogens but would also decrease the chances of these organisms developing resistance or adaptive responses (Kaufman et al., 1999; Wink, 1999; ElGengaihi et al., 1995).

Harvesting of drug from medicinal plant

Ancient medical texts, some dating back to the early Greeks, talk about medicinal plants. Now modern science--including some done by USDA's--is taking this ancient art to new levels, Plant physiologist Stephen O. Duke heads ARS' Natural Products Utilization Research

Unit at Oxford, Mississippi. Part of his research involves discovering how plants make their beneficial compounds and how to better extract them. One example of his work is understanding the plant known as annual wormwood, *Artemisia annua*. This gray-green aromatic plant and its relatives in the genus *Artemisia* have been used to make absinthe and flavored wines since earliest times. Now this plant family could bring a new gift: Its natural pest-fighting defense may protect humans from malaria.

It is no secret that malaria-fighting drugs have done a lot for civilization--the Panama canal is one testimony of their success. But what happens when the organisms that cause the disease develop resistance to current treatments? Right now, scientists are preparing to solve this problem before it ever occurs by having alternative treatments ready. One of these understudy cures could be artemisinin, a natural compound produced by *Artemisia* plants. Medical researchers, especially in the military, want to know more about wormwood's malaria-fighting properties. That's because when duty calls U.S. troops to a tropical area, the disease is always a potential problem. The question Duke wants to answer is exactly how the plant produces this potentially life-saving compound.

Knowing the physiology would play a role in increasing the supply of this beneficial compound. "It was already known that wormwood has little balloon like glands on its leaf surface," Duke says. "We found that as the plant matures, these balloons fill with artemisinin. Pest protection is nature's goal. As the plant matures, the glands swell bigger and finally burst, covering the plant with self-made pesticide. "It is the natural pesticide aspect of annual wormwood that brings it even more within Duke's expertise. His research group's primary mission is to find natural products to control agriculture pests--especially in America's secondary crops.

There is money to be made in fighting pests, but it is mainly in protecting the nation's agronomic superstars--corn, wheat and soybeans. That can mean that important, but less prominent, crops such as avocados and orchids are ripe for new protective treatments. The scientists in Duke's research unit search the plant and microbial kingdoms for pest-fighters that work in harmony with the environment. Artemisinin, for example, may also yield its protective powers to otherwise vulnerable crops. "We found that without the genetic coding for the gland, *Artemisia* will not produce artemisinin," says Duke. "For the plant, it is essential to have the genetic instructions for both the toxin and the storage mechanism. "Knowing how a plant's DNA programs it for pest protection may lead researchers to ways to provide the same pest resistance to currently vulnerable plants.

Importance of the some phyomedicinal drugs

Besides the crop protection aspects of their research, Duke and his team of scientists are also looking at the

pharmaceutical value of these plants. In fact, *Artemisia* is just one of the plants they are exploring. Another plant that's intrigued Duke is St. John's-wort, which belongs to the genus *Hypericum* (Chatterjee et al., 1995, Forensiero et al., 1995). Greek texts dating back to 2000 B.C. have noted uses and harvesting techniques for this plant. Currently, its claim to fame is as an alternative treatment for depression. "St. John's-wort (Laakman et al., 1998; Upton, 1998) is the preferred treatment for mild depression in Europe," says Duke. "Physicians there choose it four to five times more often than synthetic drugs because they believe it has fewer side effects. Europeans get their supply from Albania, but it grows wild in the United States. "Much to the chagrin of ranchers who know St. John's-wort as a pest, horses and cattle that eat the plant develop a sensitivity to light, resulting in a rash, Duke says. Understanding the biochemistry involved might help reduce the effects.

But now, however, Duke's main concern is extracting a red pigment from the plant to benefit people. St. John's-wort tablets are standardized by the amount of the red pigment, called hypericin, which some researchers suspect is the active ingredient. Hypericin is being studied as both an anti-viral and anti-cancer drug. St. John's-wort has yet to receive FDA approval for use as a treatment for depression in the United States. But several companies are doing phase I and II clinical trials, a step toward gaining approval. Currently, Americans can buy St. John's-wort as a diet supplement.

Getting the good stuff out

Finding an economical extraction method for hypericin would be helpful in developing St. John's-wort both for medicinal purposes and as an anti-viral agent. Since plant extraction and physiology are aspects of Duke's work, he began with research on the plant. It was already known that hypericin was concentrated in small black and red dots found on the flowers and leaves of St. John's-wort and that it was effective in pest control. But hypericin, if given in a high enough concentration, is toxic to all living things, including St. John's-wort (Nahrstedt and Butterwick, 1997). The plant protects itself by sealing the hypericin dots off with a thin cell layer. Normally, hypericin is extracted by chopping the plant up and extracting it with ethanol. But Duke may have a better way. "The plant has other enzymes that can destroy hypericin when the cells containing the toxin are breached," he says. "Crushing the plant releases these hypericin-destroying proteins, defeating your purpose. We are looking at chemical extraction methods that may work better--like soaking the leaves in a solution that gently removes the hypericin without having to cut them. "While breeders and pharmaceutical companies are waiting to see what this plant can do, they can also stay tuned to Duke's research team. Already these scientists are hot on the trail of new helpful plants and better ex-

traction techniques. Last fall, Duke had just finished completing his staffing and the scientists were busy ordering equipment to start new research projects.

CULTIVATION VERSUS WILD HARVESTING OF MEDICINAL PLANTS: IS CULTIVATION THE SOLE SOLUTION

Up to 90% of species of Medicinal and Aromatic Plants (MAPs) traded in Europe are still harvested from the wild and a rapid growth in the market is now resulting in over-exploitation of wild stocks of some species.

According to TRAFFIC, the wildlife trade monitoring programmer, over-exploitation threatens up to 150 species in at least one European country (Lange, 1998). Modern destructive harvesting techniques and habitat loss and alteration resulting largely from changing agricultural practices over the past 100 - 200 years, are threatening the local survival of species or varieties. The extinction of the ancient plant Silphion in about 250 BC – probably caused by its uncontrolled harvest – provides a warning of the need for sustainable management of plants while it is still possible (see Fact sheet 3: 'Use of plants for medicine around the world'). Changes in political and related socio-economic structures can place MAPs under threat. The collapse of communist regimes in former Eastern Bloc countries in Europe resulted in deregulation of state-controlled commerce and a weakening of pre-existing quota-controlled harvesting structures. As a result, the number of traders in MAPs has increased and wild collection has grown in an uncoordinated fashion (Lange, 1998). This has not necessarily benefited the traditional harvesters or the continuation of the stocks on which they have depended.

CULTIVATION

Out of about 2,000 MAPs traded in Europe, 1,200 - 1,300 are native to the continent with only 130 - 140 species predominantly derived from cultivated stock. An estimated 70,000 ha of land are devoted to the cultivation of MAPs in the European Union. France, Hungary and Spain have large areas under cultivation (Lange, 1998), major species including lavender (*Lavandula* spp.), opium poppy (*Papaver somniferum*), caraway (*Carum carvi*), fennel (*Foeniculum vulgare*) and peppermint (*Mentha x piperita*).

Other countries with large-scale cultivation include Argentina, Chile, China, India and Poland (Kuipers, 1997; Huang, 1999). Cultivation is commercially attractive to companies because they have greater control over quality and supply. Its feasibility depends on a species' ability to thrive as a monocrop, while its economic viability depends on the volumes required and market prices. MAPs likely to be cultivated are those that are

high yielding and responsive to economies of scale. Slow-growing, space-demanding, or low-yielding species are less likely to be economically attractive to commercial growers. Cultivation is particularly advantageous for growers where there are long-standing partnerships and contractual arrangements to supply manufacturers. Domestication of a species can be difficult and expensive.

As a result, wild harvesting is likely to remain the only option for many species. Wild harvesting is generally much cheaper than expenses incurred in the establishment of cultivation, a process which may take 20 years (Kuipers s.a.; Lange, 1998). Cultivation of MAPs is a promising means of meeting an expanding market while reducing pressure on populations of wild plants (Lange, 1998). Cultivation can have the potential to save wild populations of MAPs, thus, contributing towards conservation of their genetic diversity. Conservation of genetic diversity can also be achieved through *ex situ* efforts involving botanic gardens and seed banks, preferably in combination with *in situ* measures.

WILD HARVESTING

While many MAPs are common in the wild and can be freely harvested without undue concern, some of the most popular and most traded are under severe threat from over-harvesting. They face commercial or local extinction, if not necessarily global extinction (Lange, 1998). Wild-harvesting of MAPs in Europe is still prominent in Hungary, Spain, Turkey and many former Eastern Bloc countries such as Albania, the Czech Republic and Poland. Between 20,000 and 30,000 tones of wild-plant material are collected annually in Europe (Lange, 1998) and more than 500 MAPs were harvested from the wild in France during 1988-89 (Kuipers s.a., Bruneton, 1995). Between 30 and 50% of MAP material in trade in Hungary is wild-collected, the equivalent figures for Germany being 50 – 70%, 75 – 80% for Bulgaria and almost 100% for Albania and Turkey (Lange, 1998).

Trade in MAPs is largely unmonitored and harvesting practices are frequently unsustainable. It is rare for there to be effective controls over the amounts harvested. In general, there is little knowledge of the ecology and regeneration abilities of many MAPs and accordingly, little understanding of steps necessary to ensure the maintenance of populations [hyperlink to Fact. Sheet 1: 'Towards sustainable herbal medicine?']. An advantage in maintaining wild populations of a species, not least through their sustainable harvesting, is that reserves of genetic variability remain. These can also be useful for reinvigorating cultivated stock. It can be assumed that each individual wild population will normally be quite genetically diverse. The sum total of all the wild populations of a species, across its geographical range, constitutes an invaluable resource.

Wild populations frequently contain genes of value in plant-breeding – for instance to increase the levels of active principles or to convey resistance to disease. Countries can protect their wild gene-pools of MAPs by working with corporate users to introduce policies that link controlled harvesting with habitat management and monitoring. Such policies may be difficult to enforce across the entire territories of countries and it is suggested that measures be introduced at trial sites and then expanded. In Spain, almost 100% of thyme (see also Fact sheet 1: 'Towards sustainable herbalmedicine') comes from wild plants, mainly growing in the South-east, where most of the companies dealing in this commodity are situated (Lange, 1998). The herb is harvested in May and June by uprooting whole plants, often using machines, with the result that entire hillsides are stripped.

Disturbance to the topsoil and clearance of vegetation cover make slopes vulnerable to erosion. Furthermore, the removal of whole plants destroys rootstocks, so that they become unavailable as sources of new shoots. Opinion is divided as to whether this practice is damaging: some say it stimulates regeneration from seed, although this still leaves the collateral damage of erosion. Possibly, the extent of erosion is dependent on the degree of disturbance. Harvesting could be made more sustainable by just cutting the branches. Furthermore, increased cultivation would reduce the need for wild harvesting. Weleda, a Swiss-based herbal remedies and cosmetics company, has found that Arnica (*Arnica montana*) can be harvested sustainably if only sections of the above-ground parts of the plants are removed together with only small parts of the rhizomes.

This method stimulates dormant buds in the remaining rhizomes into growth and avoids losses of whole plants or severe disturbance to the habitat (Ellenberger, 1998). Collection from the wild may be unavoidable or even preferable for those many MAPs that grow slowly, are difficult to domesticate or for which only small quantities are needed. The cost of wild-collection is typically much less than that of cultivation. Risks associated with wild collection include:-

- i. Over-harvesting of endemic MAPs – species with very restricted geographic distributions can be vulnerable to extinction.
- ii. Loss of genetic diversity through the reduction or elimination of local plant populations with unique genetic characteristics.
- iii. The unnecessary destruction of plants resulting from careless and unsophisticated harvesting practices (Harnischfeger, 2000). Some practitioners and patients prefer to use wild stock because they believe in the greater efficacy of wild-harvested material.

To investigate this, the University of Westminster has established a programme, Herbs at Highgrove, to study herb-growing in Britain. Initial findings indicate a loss of

efficacy in cultivated stock. These results mirror a conclusion reached through research by the herbal company Weleda with reference to Arnica (*Arnica montana*). Arnica thrives in poor meadows with acidic soils in mountainous areas of Europe. It is listed as endangered in the Red Data Books for Germany and Hungary (Lange, 1998).

In cultivation trials a selected cultivar initially showed promise (Ellenberger, 1998), but analysis of its biochemical properties revealed differences with wild populations. Although the cultivated stock grew vigorously, their rhizomes were found to have lost much of Arnica's characteristic smell and taste, reducing its commercial potential. A search was then made for a better strain, which was eventually successful. This example demonstrates that conservation of wild populations is needed to identify varieties for cultivation and as sources of useful genes. Wild-harvested material can be much higher-priced and sought after. For example, the cultivation of American ginseng (*Panax quinquefolius*) might not save this species (Li and Mazza, 1999; Attele et al., 1999) from extinction in the wild, because Asian buyers will pay up to 30 times more for wild-harvested roots, which is a huge incentive for collectors. On the other hand, it is easier to ensure that standards of quality are maintained, including given levels of active compounds, with cultivation. Also, it is easier to control post-harvest treatment, providing buyers with consistent products.

WHAT ARE THE SOCIAL BENEFITS OF WILD HARVESTING OR LOCAL MULTICROPPING SYSTEMS?

Harvesting MAPs from the wild is generally a seasonal activity, providing a supplementary income for rural people such as stock-herders, women, children and the retired – generally the poorest people in communities (Lange, 1998). On the other hand, small-scale cultivation of MAPs, often in multi-cropping systems, can be a means by which low-income families can boost their earnings.

In Guatemala, the German Ministry for Economic Cooperation and Development (BMZ) has shown that medicinal plants can be integrated successfully into traditional farming systems with food-crops such as maize, beans and vegetables and that they can provide a regular income (Eid, 2000). Where harvesting is regulated by custom or law, wild-harvesting can be sustainable and of benefit to conservation, as well as maintaining sources of income for local people.

At village level in many developing countries and more traditional societies, medicinal plants can provide some of the strongest links between people and nature. Maintaining such links through the sustainable harvesting of MAPs can be a crucial factor to ensure the continua-

tion of those traditional cultural landscapes in which MAPs thrive, such as traditionally-managed Alpine meadows or the Hungarian Steppes. The conservation of vulnerable habitats and species by designation of parks or reserves can attract tourists and provide jobs to local people with few other opportunities for regular employment. Plants such as bog bean (*Menyanthes trifoliata*) could be protected in this way.

Wetlands are disappearing across the world and bog bean is becoming increasingly scarce as its wetland habitat is drained. Cultivation of MAPs does not necessarily result in gains for conservation and social welfare, particularly in poorer countries. In order to cultivate MAPs, growers need to have secure tenure of their land, spare land for planting and be able to afford necessary inputs which can include machinery and agrochemicals. Richer members of communities are therefore most likely to benefit, while the poor have little option but to continue harvesting from the wild – though perhaps with even less benefit. Wild harvesting of MAPs provides cash income for the poorest sectors of society, especially in non-industrialized countries.

However, prices paid to these people for the material collected are generally lower than for cultivated material (Lange, 1998). Wild-harvesters sometimes have the additional problem of little certainty that traders will return regularly to purchase their harvests. Fairer prices for collectors and incentives for better management are needed (see Factsheet 3: 'Use of plants for medicine around the world'). Fair trade schemes now being adopted in Europe and North America can contribute to fairer prices and better management.

CONCLUSION

Is cultivation a viable alternative to wild harvesting of maps?

This is not an either/or situation, but more a case of developing structures that support existing sustainable practices of wild harvest, leading to recognition of the contributions that can be made by both wild harvesting and cultivation. Wild-growing plants are sources of genetic diversity needed by the MAP industry for developing new cultivars. Furthermore, many MAPs are unsuited to high-volume mono-cropping, or the market for them is too small to be worth the expense of introducing large-scale cultivation. On the other hand, demand for MAPs can often only be met through extensive cultivation. Both wild harvesting and cultivation have social dimensions. Taken together, locally-based cultivation and wild harvesting can be effective means of providing income for the poorest sectors of society and contribute to social stability, while supporting conservation. Progress will be largely dependent on support by governments and industry.

Conclusion and outlook

For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities and plant physiological studies would indeed have a major role to play in this burgeoning field. With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. Although active phytochemicals may have been identified, in general, many pathways for the biosynthesis of specific medicinal compounds and the factors (biotic and abiotic) regulating their production remain unclear.

At present, a major concern with the use of phytomedicines regards the maintenance of consistent medicinal quality in botanical medicines (Matthews et al., 1999; Dewick, 1997; Schulz, 1998). Whereas the focus has tended to be on quality control in herbal manufacturing practices, variation in phytomedicinal content due to environmental effects upon secondary plant metabolism in the plant material could represent a significant factor. It is clear that understanding how environmental factors affect phytomedicinal production will be of great importance toward optimizing field growth conditions for maximal recovery of phytomedicinal chemicals.

Transgenic genes could also be introduced to modify existing pathways. Mutational alterations and analysis could also be performed to dissect out basic components of metabolic pathways. The application of molecular approaches with medicinal plants would also benefit from the development of cell, tissue and organ culture systems for *in vitro* growth and regeneration of medicinal plants. In addition, such tissue culture systems could also prove useful for large-scale biotechnological production of medicinal plant phytochemicals (World Health Organization, 1999). Overall, metabolic engineering could be useful for modifying or enhancing synthesis of valuable therapeutic agents present in medicinal plants. However, as the beneficial actions of medicinal plants can be related to combinations of phytochemicals acting collectively or synergistically, alteration of single phytochemical components could potentially affect the efficacy of a phytomedicine.

In this respect, any work on modification of the phytomedicinal chemical composition of a medicinal plant through molecular methods would need to be conducted in conjunction with pharmacological studies on drug effectiveness.

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