[Frontiers in Bioscience 11, 2354-2370, September 1, 2006]

Metabolism and Ecology of Purine Alkaloids

Ana Luisa Anaya [1], Rocio Cruz-Ortega [1] and George R. Waller [2]

[1]Departamento de Ecologia Funcional, Instituto de Ecologia, Universidad Nacional Autonoma de Mexico. Mexico DF 04510, [2]Department of Biochemistry and Molecular Biology, Oklahoma State University. Stillwater, OK 74078, USA

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Classification of alkaloids
4. The importance of purine in natural compounds
5. Purine alkaloids
5.1. Distribution of purine alkaloids in plants
5.2. Metabolism of purine alkaloids
6. Biosynthesis of caffeine
6.1. Purine ring methylation
6.2. Cultured cells
7. Catabolism of caffeine
8. Caffeine-free and low caffeine varieties of coffee
8.1. Patents
9. Ecological role of alkaloids
9.1. Herbivory
9.2. Allelopathy
9.2.1. Mechanism of action of caffeine and other purine alkaloids in plants
10. Perspective
11. Acknowledgements
12. References

1. ABSTRACT

In this review, the biosynthesis, catabolism, ecological significance, and modes of action of purine alkaloids particularly, caffeine, theobromine and theophylline in plants are discussed. In the biosynthesis of caffeine, progress has been made in enzymology, the amino acid sequence of the enzymes, and in the genes encoding *N*-methyltransferases. In addition, caffeine-deficient plants have been produced. The ecology of purine alkaloids has not proved to be particularly promising. However, advances have been made in insecticidal and allelopathic fields, and in the role of microorganisms play in the changes that these compounds undergo in the soil. Caffeine inhibits cell plate formation during telophase throughout the development of coffee plants and other species.

2. INTRODUCTION

Alkaloids are one of the most diverse groups of secondary metabolites found in living organisms. They have many distinct types of structure, metabolic pathways, and ecological and pharmacological activities. Many alkaloids have been used in medicine for centuries, and some are still important drugs. Alkaloids have, therefore, been prominent in many scientific fields for years, and continue to be of great interest today (1). Humans have used alkaloids from the origins of civilization, as drugs, potions, medicines, teas, poultices, and poisons (1). Therapeutically active alkaloids were isolated in early 19th century. The first alkaloid to be investigated chemically was opium, the dried latex of the opium poppy *Papaver somniferum* (Papaveraceae). Opium was used as a drug for

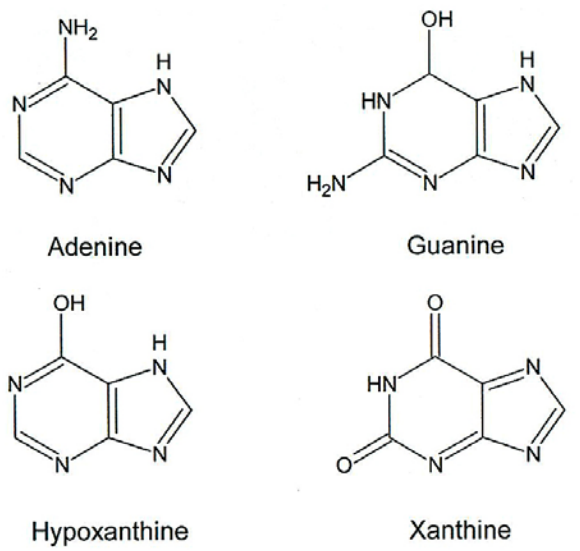

Figure 1. Structure of purines: adenine (6-amino purine), guanine (2-amino-6-oxy purine), hypoxanthine (6-oxy purine), and xanthine (2,6-dioxy purine).

centuries for its analgesic and narcotic properties. Between 1817 and 1820 the laboratory of Pelletier and Caventou at the Faculty of Pharmacy in Paris isolated many active principles of pharmaceutical importance (strychnine, emetine, brucine, piperine, caffeine, quinine, cinchonine, colchicine) (2). Of the approximately 50,000 natural products known today, over 12,000 are alkaloids (1).

This review aims to provide up-to-date information on dynamic interrelationships in plants that produce purine alkaloids (primarily caffeine, theobromine, and theophylline).

3. CLASSIFICATION OF ALKALOIDS

Pelletier (2) proposed the definition that an alkaloid 'is a cyclic compound containing nitrogen in a negative oxidation state which is of limited distribution in living organisms'. This definition includes alkaloids with nitrogen as part of a heterocyclic system and the many exceptions with extracyclic bound nitrogen, such as colchicine or capsaicin (1).

Alkaloids can be classified biogenetically. For example, the indole alkaloids derive from tryptophan, and may be subgrouped either as nonterpenoid or terpenoid indoles (iridoid). Not all alkaloids are strictly amino acid derived, and it is possible to distinguish four groups:

1. Alkaloids derived from amino acids such as ornithine/arginine, lysine, histidine, phenylalanine/tyrosine, tryptophan, anthranilic acid, and nicotinic acid.

2. Purine alkaloids, such as caffeine, theobromine, and theophylline.

3. Aminated terpenes, e.g. aconitine (a diterpene) and solanine (a triterpene).

4. Polyketide alkaloids in which nitrogen is introduced into a polyketide carbon skeleton, as in coniine and the coccinellines (1).

Historically, the main source of alkaloids has been the flowering plants Angiospermae, of which about 20% contain alkaloids. Many alkaloids have recently been isolated from animals, insects, marine organisms, microorganisms, and lower plants.

4. THE IMPORTANCE OF PURINE IN NATURAL COMPOUNDS

Purine is the most widely distributed nitrogen heterocycle in nature. The name 'purine' (*purum uricum acidum*) was coined by Emil Fischer, who first synthesized this colorless crystalline weak base in 1899. The International Union of Pure and Applied Chemistry (IUPAC) acknowledges the importance of the purine system by the traditional name purine rather than the systematic name imidazo[4,5-*d*]pyrimidine (3). Purine represents a unique basic heterocyclic system that is unique in its chemodiversity. Unsubstituted purine does not exist in nature. Many purine derivatives, especially adenine derivatives, are involved in diverse metabolic processes. Adenosine 5'-triphosphate is used in the storage of energy in all living cells. Adenosine 3',5'-cyclophosphate (cyclic-AMP, cAMP) acts as a so-called second messenger controlling the activation of protein kinases and K^+ levels in cells, as well as in transcription and other metabolic processes. Nicotinamide adenine dinucleotide (NAD, NAD^+) and flavin adenine dinucleotide (FAD) are coenzymes involved in cellular reduction-oxidation processes. A further purine-containing molecule of biological note is acetyl-coenzyme A, which has high C_2-group-transfer potential (3).

Rosemeyer (3) asked the question, "why has nature chosen purine as the basic heterocyclic motive for so many structural variations in purine derivatives?" Rosemeyer observed that 6-aminopurine (adenine; see Figure 1), rather than purine itself, is the basic structure involved in most naturally-occurring compounds.

5. PURINE ALKALOIDS

Methylxanthines and methyluric acids are secondary metabolites derived from purine nucleotides, and are known collectively as purine alkaloids (Table 1, Figure 2). Caffeine (1,3,7-trimethylxanthine is the most-important purine alkaloid in coffee beans (2.5 wt-%) and in tea leaves (5 wt-%). Caffeine was first isolated in 1819 by F. F. Runge, the inventor of paper chromatography (3). Oudry (1827) then isolated a compound from *Camellia sinensis* having similar physiological effects to caffeine. Ten years later, Jobat and Mulder (1837) identified this compound as caffeine (4). The first total synthesis of caffeine was performed in 1895 by E. Fischer.

In unroasted coffee beans, caffeine is bound to chlorogenic acid, which takes up a crescent shape to which caffeine is linked by non-covalent bonds. High

Table 1. Trivial names and different radicals in purine alkaloid structures shown in Figure 2.

Purine Alkaloids		Trivial Name	R^1	R^3	R^7 or R
1	Xanthine		H	H	H
2	7-methylxanthine	Heteroxanthine	H	H	Me
3	1,3-dimethylxanthine	Theophylline	Me	Me	H
4	3,7-dimetylxanthine	Theobromine	H	Me	Me
5	1,7-dimethylxanthine	Paraxanthine	Me	H	Me
6	1,3,7-trimetylxanthine	Caffeine	Me	Me	Me
7	1,3,7,9-tetramethyluric acid	Theacrine	-	-	-
8	O^2,1,9-trimethyluric acid	Liberine	-	-	H
9	O^2,1,7,9-tetramethyluric acid	Methylliberine	-	-	Me

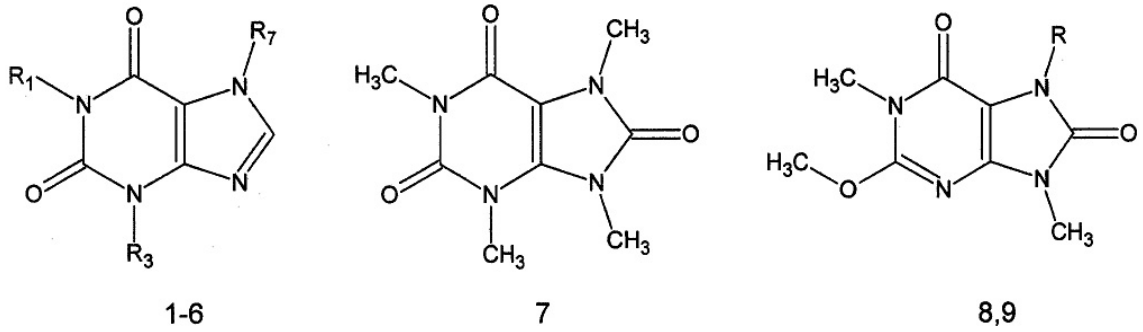

Figure 2. Structure of purine alkaloids in plants.

concentrations of purine alkaloids (mostly caffeine) accompany the accumulation of chlorogenic acids (mostly 5-caffeoylquinic acid), and vice versa. In tea leaves, caffeine is bound non-covalently to gallic acid, and ellagic acid, which are tannic acids. All caffeine-containing plants may have evolved a common strategy to sequester purine alkaloids: the vacuolar allocation of high concentrations of one or several phenols in a complex (5, 3).

The action of caffeine on the central nerve system of humans is based on its ability to inhibit a cyclo-AMP-specific phosphodiesterase, slowing the conversion of cyclic-AMP to AMP and thereby extending the effect of adrenalin-mediated cyclic-AMP. The stimulant effect of caffeine begins 15 min after consumption and lasts *ca.* 5 ± 6 h. A normal dose (100 mg) stimulates heart function, metabolism, respiration, enhances blood pressure and body temperature, and promotes the constriction of blood vessels in the intestines but vasodilatation in the brain. The improved blood circulation in the brain is effective against sleepiness. 1,7-Dimethylxanthine (paraxanthine) is the major metabolite of caffeine in humans, and is believed to contribute to the physiological adenosine antagonist effects of caffeine (6).

Some studies have, however, found that exposure to caffeine is related to poor neuromuscular development and significant increases in breech presentation of fetuses (7). Caffeine and theobromine can penetrate the placental barrier quite easily (8) and so affect the developing fetus. Consumption of caffeine during pregnancy leads to changes in the levels of brain chemicals such as DNA, zinc, cAMP, protein, and alkaline phosphatase (9, 10). For a recent review on caffeine, see Waldvogel (11).

In tea plants, caffeine is found together with theophylline (1,3-dimethylxanthine). Both substances are effective as diuretics. Theophylline is sometimes prescribed as an anti-asthma drug, particularly for use during acute asthma attacks. In the cocoa bean, theophylline is replaced by theobromine (3,7-dimethylxanthine) as the main xanthine component. Even much studied compounds such as these may act as lead structures in modern medicinal chemistry (3).

5.1. Distribution of purine alkaloids in plants

Compared with other alkaloids such as nicotine, morphine, and strychnine, purine alkaloids are widely distributed throughout the plant kingdom. Caffeine has been found in 13 orders of the plant kingdom. Most caffeine-containing plants are members of the dicotyledoneae, although *Scilla maritime* belongs to the monocotyledoneae. In some species the main purine alkaloid is theobromine or methyluric acids including theacrine (1,3,7,9-tetramethyluric acid), rather than caffeine (Table 2) (12).

Coffee is a shrub or small tree native to the mountains of Ethiopia, although it is now grown throughout tropical regions of the world. Coffee was originally roasted and used as a beverage in Arabia. Mocha, a city in Yemen, was an ancient center of coffee trading. During the 17th century coffee houses sprang up in England, where they became popular centers for political discussion and meeting places. Sri Lanka (Ceylon) was the main source of coffee imported by England, but a serious fungal disease (*Hemileia vastatrix*) destroyed the coffee plantations in 1869. England then switched to Ceylon teas, which are the most commonly brewed beverage in Britain

Table 2. Distribution of purine alkaloids in plants (mature leaves or seeds)

Latin name	Family name	Common name	Major alkaloid
Coffea arabica	Rubiaceae	Arabica coffee	Caffeine
Coffea canephora	Rubiaceae	Robusta coffee	Caffeine
Coffea liberica	Rubiaceae		Theacrine, liberine
Coffea dewevrei	Rubiaceae		Theacrine, liberine
Camellia sinensis	Theaceae	Tea	Caffeine
Camellia assamica	Theaceae	Assam tea	Caffeine
Camellia assamica var *Kucha*	Theaceae	Kucha	Theacrine
Camellia taliensis	Theaceae		Caffeine
Camellia irrawadiensis	Theaceae		Theobromine
Camellia ptilophylla	Theaceae	Cocoa tea	Theobromine
Theobroma cacao	Sterculiaceae	Cacao (cocoa)	Theobromine
Theobroma grandiflorum	Sterculiaceae	Cupu	Liberine
Paullinia cupana	Sapindaceae	Guaraná	Caffeine
Cola sp.	Sterculiaceae	Cola	Caffeine
Citrus sp.	Rutaceae		Caffeine
Ilex paraguariensis	Aquifoliaceae	Yerba maté	Caffeine

Modified with permission from Ref. 12.

to this day. About half of the world's total coffee production comes from large plantations in Brazil and Colombia. Coffee fruits are fleshy berries, each containing two seeds which are pressed together so that the inner (adjacent) side of each one is flattened. Coffee beverage is made from the ground, roasted seeds (coffee beans) that are removed from the coffee berries (coffee cherries). Ashihara and Suzuki (12) reported that the caffeine content of seeds of different *Coffea* species varies from 0.4 to 2.4% dry weight. Mature leaves of *C. liberica, C. dewevrei* and *C. abeokutae* contain theacrine, liberine, and methylliberine.

Green and black teas are caffeine beverages from *Camellia sinensis*, an Asian shrub closely related to ornamental species of *Camellia*. In green tea the leaves are dried and appear dull green. In black tea the leaves are fermented and then dried. "Oolong tea" is partly fermented and is intermediate between black and green. The various pekoes, souchongs, and congous are black teas, while gunpowder tea and hyson tea are the most important grades of green tea. Nagata and Sakai (13) reported that the caffeine content of young leaves from shoots of *C. sinensis* var. *sinensis, C. sinensis* var. *assamica,* and *C. taliensis* were 2-3%, and that of *C. kissi* was less than 0.02%. Theobromine is the predominant purine alkaloids in *C. irrawadiensis* (<0.8%). Zheng *et al.* (14) found that theacrine (1,3,7,9-tetramethyluric acid) and caffeine are the major purine alkaloids in the leaves of an unusual Chinese tea known as Kucha (*Camellia assamica* var. *kucha*). Endogenous levels of purine alkaloid in expanding buds made up of ca. 40% caffeine, 40% theacrine, and 20% theobromine. In young leaves caffeine and theacrine represent 15% and 75% respectively, and in mature leaves caffeine and theacrine represent 34% and 60% respectively. Radioactivity experiments indicate that, in kucha leaves, theacrine is synthesized from caffeine in what is probably a three-step pathway, with 1,3,7-methyluric acid acting as intermediate.

Yerba maté is a popular caffeine beverage in South America. In Argentina, Uruguay, and Paraguay a small gourd is made into a special cup for drinking "yerba maté," a popular tea brewed from the leaves of a native holly (*Ilex paraguariensis*). Maté is sipped through a perforated metal straw called a "bombilla." Old maté gourds improve the flavor of this caffeine-rich tea, and maté connoisseurs would never think of using glass, pottery mugs or styrofoam cups. Ashihara and Suzuki (12) reported that the purine alkaloid content of maté is: caffeine 3-4%, theobromine 0.08 to 0.16%, and theophylline <0.02%.

Of the few purine alkaloid-containing genera that are consumed as stimulants, *Paullinia* (guaraná) is the least investigated, in its chemotaxonomy and within-plant allocation of caffeine and its allies. *Paullinia cupana*, the cola of Brazil, is a high-caffeine (4-5%) beverage made from the seeds of a trailing shrub or vine native to central Brazil. The drink contains more caffeine than coffee or tea, and a single cup is reputed to be sufficiently stimulating to counteract feelings of fatigue. The powdered seeds are also available in tablet form. Since purine alkaloids are valuable marker compounds in chemotaxonomy, Weckerle *et al.* (15) screened for them 34 species of *Paullinia* and related genera. Only one, *P. pachycarpa*, was positive in caffeine production, in addition to the already known *P. cupana* and *P. yoco*. Purine alkaloid allocation in *P. pachycarpa* was examined and was found to be restricted to theobromine in the stem, leaves and flowers. The theobromine concentration in the stem cortex increases significantly towards the base of the plant. Since the stem cortex of *P. yoco* is traditionally used by the natives of Colombia and Ecuador to prepare a caffeine-rich beverage, it is likely that, within the genus *Paullinia,* purine alkaloids are preferentially allocated to the older parts of the stem and not to young shoots as in the coffee plant (*Coffea* spp.).

The cola shrub is native to the African countries of Ghana and Nigeria. The cola seed (cola nut) is rich in caffeine, and is used in many popular cola beverages. In western Africa the cola nut is chewed by native people to inhibit fatigue and hunger. The original Coca Cola® was made from cola beans and coca leaves (*Erythroxylum coca*). Today's Coca Cola contains decocainized coca leaves.

Table 3. Caffeine, theobromine and theophylline content of individual coffee fruits at different stages of growth

Age (months)	Growth stage	Alkaloid levels (μg g^{-1} dry wt)		
		Caffeine	Theobromine	Theophylline
0.3	Green	9200	146	-
1	Green	8000	145	-
4	Green	7900	55	-
5-6	Yellow-red	7500	59	28
7-8	Brown-black	6100	56	56

Modified with permission from Ref. 19.

The chocolate or cocoa tree (*Theobroma cacao*) is believed to have originated in the Amazon Basin on the eastern equatorial slopes of the Andes. It is a small, shade-loving understory tree of wet tropical lowland forests. Many Indian tribes believed the plant came from the gods, hence the generic name *Theobroma*, meaning "food of the gods." In fact, it was so revered by native people that the seeds were used as currency by Aztecs and Mayas. The cauliflorous inflorescences grow directly from the trunk and branches. The large, oblong fruits contain five rows of large seeds which are roasted and processed into cocoa. Ashihara and Suzuki (12) state that the seeds contain the alkaloid theobromine (2.2% to 2.7%), which is a caffeine relative with many reputed attributes, from a mild stimulant to a pleasant aphrodisiac. Cocoa also contains caffeine in lesser concentration (0.6%). Seeds of eleven *Theobroma* species and nine *Herrania* species contained 0.25% liberine in cotyledons.

Members of the genus *Citrus* as well as the related genera *Poncirus* (e.g. Trifoliate Orange), *Fortunella* (e.g. Kumquat), *Murraya* (e.g. Satinwood) have the common feature of producing caffeine and related purine alkaloids. However, these compounds accumulate in only a few tissues or organs, and the rest of the plant is virtually free of purine alkaloids. Kretschmar and Baumann (16) found purine alkaloids in the male part of *Citrus* flowers, the stamina. They followed the formation and accumulation of purine alkaloids during development of the flowers of lemon, canton lemon, and trifoliate orange cultivars, and found the greatest concentrations at the time of full opening (anthesis) of the flower; in contrast, the flower bud contains no purine alkaloids. In the opened flower, the filament, anther and pollen all accumulate caffeine and, to a lesser extent, theophylline. These authors also observed the allocation of purine alkaloids exclusively in the male part of the flower in cultivated cocoa (*Theobroma cacao*) and "wild" cocoa (*T. grandiflorum*); the (non-fertile) staminoids contained no purine alkaloids. The stamina of the staminate flower of a "wild" cola (*Cola humilis*) likewise accumulates this type of alkaloid, whereas the stunted stamina of the pistillate flower accumulates almost none. Since such high concentrations of purine alkaloids are toxic to a wide array of organisms, this specific allocation to the nutrient-rich stamen is clearly to keep predators away. Interestingly, in contrast to many other insects, the honey bee is caffeine-tolerant. Kretschmar and Baumann (16) also state that orange flower tea (Aurantii flos) is recommended pharmaceutically for treatment of sleeplessness. The amount of caffeine ingested by consuming such a calming tea is typically below 100 mg, a dose present in homeopathic coffee preparations used against insomnia.

5.2. Metabolism of Purine Alkaloids

Weevers (17) showed that xanthine derivatives are found in coffee, tea, maté, guaraná, cocoa and some lesser known species of plants that have a biochemical relation to each other, based on similarity of the compounds. He attempted to relate the analytical chemistry of these compounds and the plants that produced them. However, he was not able to put them together in a satisfying single metabolic pathway. Weevers did recognize that the plants were from widely geographical regions of the world, and that their physiology was unique.

Plants biosynthesize alkaloids from simple precursors using many unique enzymes that utilize widely distributed compounds, including L-amino acids (tryptophan, tyrosine, glutamine, aspartate, etc.) and terpenoid derivatives. The *de novo* formation of purine nucleotides in plants is not well understood, but is thought to involve a pathway that is defined in other organisms. In a complicated metabolic pathway, glutamine and aspartate are important intermediates; however, inosine monophosphate (IMP), the first product with a complete purine ring, can serve as the precursor of xanthosine-5-monophosphate (XMP), guanosine-5-monophosphate (GMP), and adenosine-5-monophosphate (AMP). There are five distinct pathways that operate in caffeine biosynthesis; a common one in coffee and tea is the AMP $\rightarrow$ IMP $\rightarrow$ XMP $\rightarrow$ Xanthosine pathway (12). These multiple pathways involving purine nucleosides and leading to the formation of xanthosine allow plants that produce caffeine a wide variety of opportunities to biosynthesize caffeine, depending on where they grow and under what conditions (18).

Metabolism of purine alkaloids (caffeine, theobromine, and theophylline) occurs in coffee, tea, maté, cocoa, and guaraná; however, most research has been done with tea and coffee plants. Purine metabolism is similar to that in other plants which do not contain caffeine. Young leaves of *Coffea arabica* contain about 1-2 % caffeine (dry weight) whereas young tea leaves (*Camellia sinensis*) contain 2-4 % caffeine. Mature and aged leaves of both species contain lower quantities of caffeine.

Table 3 shows the pattern of formation of caffeine, theobromine and theophylline in coffee fruits during the growth stages of the bean (19). The caffeine concentration was highest at 0.3 months, after which it dropped steadily during the 7-8 month growth period. The theobromine concentration peaked at 0.3-1 months and then remained fairly constant. In contrast, theophylline was present only through out the catabolism period; levels were

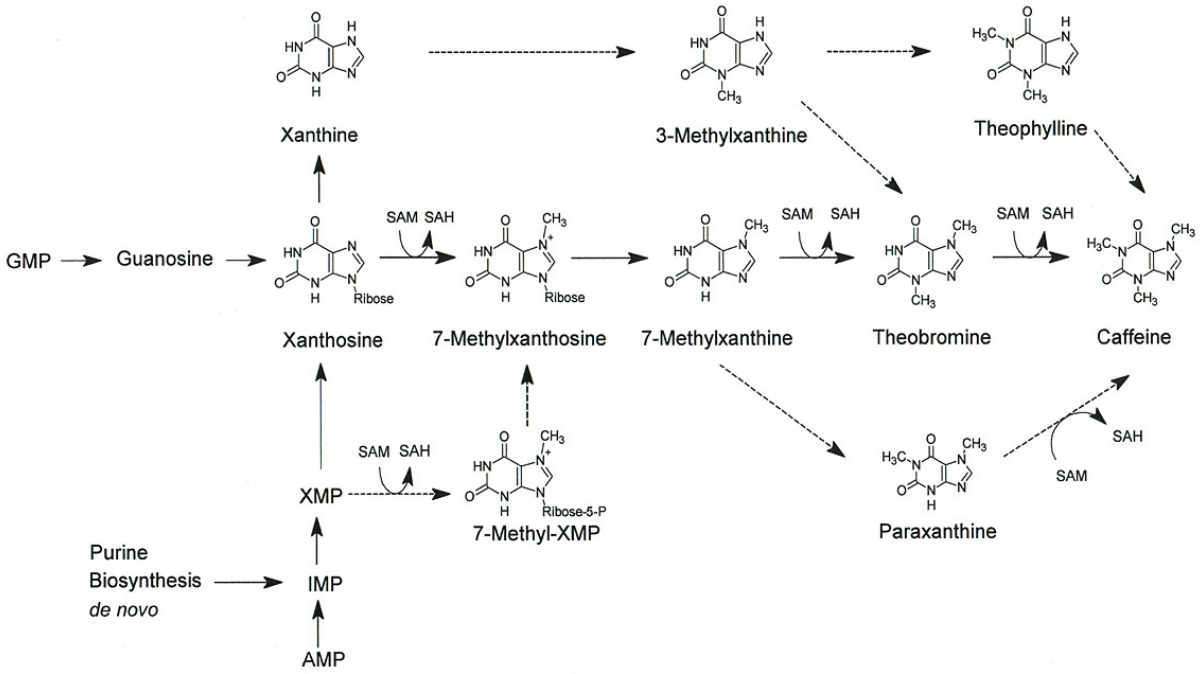

Figure 3. Key pathways for the biosynthesis of caffeine. Abbreviations: GMP, guanosine 5'-monophosphate; XMP, xanthosine 5'-monophosphate; IMP, inosine 5'-monophosphate; AMP, adenosine 5' monophosphate; SAM, *S*-adenosyl-L-methionine; SAH, *S*-adenosyl-L-homocysteine. Reproduced with permission from Refs. 43 and 55.

near zero until a small increase at 5-6 months. Theophylline increased in the yellow-red period and approximately doubled during the 7-8 month period. Theophylline never reaches the concentration of caffeine, but it does correspond to the amount of theobromine during the 5-9 month old stages. Paraxanthine was not found in *C. arabica* fruits during their development, although it has been reported in young coffee seedlings (20), and callus cultures (21).

Roberts and Waller (20) used 2-3 and 7-8 month old seedlings of *C. arabica* obtained from the Royal Botanic Gardens, Kew, England, and 2-3 month old seedlings from Kings College, London, and found that the young seedlings were quite active biosynthetically relative to older leaves and fruits.

Zheng and Ashihara (22) suggest that caffeine accumulation is specific to the above ground parts (leaves, cotyledons, and shoots) of the seedlings, and that biosynthesis is performed only in these very young tissues; Waller *et al.* (23) showed that the soil under the coffee tree has extremely high concentrations of caffeine, falling with soil depth to 3 ft. below the surface. The soil is in contact with the roots of the plant at all times. This high concentration of caffeine in the soil produces an allelopathic effect which will be discussed in section 9.2.

Coffee cherries displayed the greatest biosynthetic activity in the young stages; however, when the fruit turns red (which is picking time) the biosynthetic activity is much lower. Catabolism begins to occur as the cherry turn red-brown-black (23). We are not aware of any study that relates the harvest of coffee beans with their colors, from green-orange-red-brown-black to caffeine production; however, the coffee cherries are harvested upon turning red.

During the last 40 years, almost complete metabolic pathways of caffeine have been deduced, largely in the work of Roberts and Waller (20), Waller *et al.* (23), Baumann and Frischknecht (24, 25), Baumann (26), Ashihara *et al.* (27, 28, 29, 30); Ashihara and Crozier (31, 32, 33), Ashihara and Suzuki (12), Mazzafera *et al.* (34, 35) and Mazzafera (36, 4). Metabolic pathways that regulate caffeine in the tissues of coffee and tea have been studied by Ashihara and Crozier (31, 32, 33), Ashihara and Suzuki (12), Koshiishi *et al.* (37), Kato *et al.* (38), Ogawa *et al.* (39), and Suzuki *et al.* (40). There is general agreement that theobromine is the immediate precursor of caffeine and theophylline is the primary metabolic product, although differing species of *Coffea* and *Camellia* may vary in their order of biosynthetic precursors or biodegradation products (41, 42, 43). *Ilex paraguariensis* (maté), *Theobroma cacao* (cocoa), and *Paullinia cupana* (guaraná) have similar precursors or degradation products; however they all contain purine alkaloids that make them useful as different types of food and drinks worldwide.

6. BIOSYNTHESIS OF CAFFEINE

Caffeine is a trimethylxanthine, with the xanthine skeleton derived from purine nucleotides that are converted to xanthosine, which serves as a committed intermediate in the caffeine biosynthesis pathway. There are at least four purine nucleotides that play key roles in the formation of xanthosine, as shown in Figure 3 (AMP, IMP, XMP, and GMP). The evidence suggests that the most important

routes are the production of xanthosine from inosine-5'-monophosphate (IMP), which is derived from the *de novo* purine nucleotide biosynthesis (42). Adenosine which is released from *S*-adenosyl-L-homocystine (SAH) is converted to adenine, adenosine-5'-monophosphate, inosine-5'-monophosphate, and xanthosine-5-monophosphate (31, 32, 33, 12, 22). Coffee and tea have the same key pathways of caffeine biosynthesis.

Caffeine formation is closely associated with the S-adenosyl-L-methionine (SAM) cycle, known as the activated-methyl cycle. There are three methylation steps in the formation of caffeine that use SAM as the methyl donor. SAM is converted to S-adenosyl-L-homocysteine (SAH) in these processes, which is then hydrolyzed to L-homocysteine and adenosine. The adenosine is used as a biosynthetic intermediate for caffeine, whereas L-homocysteine is recycled to replenish SAM levels. Since 3 moles of SAH are produced from the SAM cycle for each mole of caffeine biosynthesized, this pathway is capable of being the sole source of the purine skeleton and of the methyl groups needed for the biosynthesis of caffeine in young tea leaves (37).

6.1 Purine ring methylation

The main initial purine compound in the biosynthesis of caffeine is xanthosine, which acts as a substrate for the methyl groups donated by S-adenosyl-L-methionine (SAM). Using leaf discs of young coffee and tea plants, tracer experiments with labelled precursors have shown that the major route to caffeine is xanthosine → 7-methylxanthosine → 7-methylxanthine → theobromine → caffeine (Figure 3). There are several other minor pathways that can operate: 7-methylxanthosine → 7-methylxanthine → paraxanthine → caffeine; and xanthine → 3-methylxanthine, which may be converted either to theobromine or theophylline → caffeine (43). Another pathway in coffee leaves is XMP → 7-methyl XMP → 7-methylxanthosine (44), but not in tea leaves (45, 46). Xanthosine is converted to xanthine, which decomposes to give CO_2 and NH_3 by the purine catabolism pathway; there are competing pathways for this important compound.

The conversion of xanthosine to 7-methylxanthosine is catalyzed by 7-methylxanthosine synthase which is a *N*-methyltransferase. This enzyme has been extracted from tea and coffee leaves (45, 46, 47), and exhibits high substrate specificity for xanthosine as the methyl group acceptor and for SAM as the donor. These authors (45, 46, 47) used partially purified *N*-methyltransferases; however, they were successful in showing that the mixture had two proteins. Xanthosine-7-*N*-methyltransferase (XMT) and its corresponding gene have been claimed to be isolated from coffee leaves (48, 49). However, its sequence does not resemble that of present CaXMT1, but rather a lipolytic enzyme (59). The same results have been obtained by another group (55).

Schulthess *et al.* (44) proposed a XMP pathway starting with the methylation of XMP. However, recent molecular biology studies do not support this pathway (59), although Mizuno and collaborators (55) could not exclude the possibility that unidentified *CtCSs* are related to the pathway proposed by Schulthess *et al.* (44).

Methylxanthine nucleosidase catalyses the hydrolysis of 7-methylxanthosine to 7-methylxanthine, with ribose given off; this enzyme is the second involved in the formation of caffeine (50). Using crude extracts from tea leaves, Suzuki and Takahashi (51, 52) showed that the *N*-methyltransferases can catalyze the conversion of 7-methylxanthine to theobromine and of theobromine to caffeine.

Mazzafera *et al.* (35) reported an *N*-methyltransferase from coffee fruits and leaves of *C. arabica* which displayed 7-methylxanthine and theobromine *N*-methyltransferase activity, like 7-methylxanthosine synthase. Kato *et al.* (46) have reported the activities of *N*-methyltransferases in caffeine synthesis. The present enzyme proved difficult to isolate and purify, although Kato *et al.* (53), using young tea leaves, were able to purify to apparent homogeneity an *N*-methyltransferase which was called caffeine synthase. It displayed both *N*-3 and *N*-1 methyl transferase activities, and high levels of substrate activity with substrates as broad as paraxanthine, 7-methylxanthine, theobromine, but low activity with 3-methylxanthine and 1-methylxanthine. Caffeine synthase has no 7-methyl xanthosine synthase activity toward either xanthosine or xanthosine-5-monophosphate. The highest V*max*, K*m* values of caffeine synthase are for paraxanthine, which is the preferred substrate (53). Roberts and Waller (20) were the first to show that paraxanthine is the preferred route of biosynthesis of caffeine in very young coffee plants. There is only limited biosynthesis of paraxanthine from 7-methylxanthine, leading to the conclusion that it plays only a limited role as a methyl acceptor in the caffeine biosynthetic pathway in growing and mature plants.

Mizuno *et al.* (54, 55) recently identified and characterized the genes responsible for encoding the enzymes for the methylation of caffeine biosynthesis. Ashihara's group in Tokyo (which includes Mizuno) has performed outstanding research on cloning the transmethylase enzymes leading to biosynthetic activity of caffeine. In outline, they have found genes encoding caffeine synthases [CtCS], such as coffee theobromine synthase CTS1, CtCS2 and CCS1 that were cloned. CTS1 and CTS2 are homologous genes encoding theobromine synthases from young coffee leaves. Independently, Ogawa *et al.* (39) and Mizuno *et al.* (54, 55) made several clones of coffee *N*-methyltransferase genes using the sequence of the tea caffeine synthase (TCS) gene isolated by Kato *et al.* (38).

N-methyltransferases involved in the caffeine biosynthetic pathway have been cloned and characterized from coffee, and designated as *CaXMT1*, *CaMXMT1, CaMXMT2,* and *CaDXMT1*. The bacterially expressed encoded proteins were characterized for their catalytic properties. CaXMT1 catalyzed the formation of *7*-methylxanthosine from xanthosine. CaMXMT2 catalyzed the formation of 3,*7*-dimethylxanthine (theobromine) from

7-methylxanthine; and CaDXMT1 catalyzed the formation of 1,3,*7*-trimethylxanthine (caffeine) from 3,*7*-dimethylxanthine (38, 39, 54, 55, 56, 57, 58, 59).

Ogita *et al.* (58) have applied RNAi to repress the gene encoding theobromine synthase (CaMXMT1) and produce transgenic coffee plants. They found that in coffee plants the major pathways of caffeine synthesis are mediated by theobromine synthase. Furthermore, the RNAi and the regeneration system developed by this group allowed the production of genetically modified coffee plants with a low level of caffeine, with promising commercial use (57).

6.2. Cultured Cells

Purine alkaloid formation in tea callus tissue cultures was first reported by Ogutuga and Northcote (60, 61), and by Tsushida and Doi (62). Using *in vitro Coffea arabica* cultures, Keller *et al.* (63) made the first observation of caffeine production. Caffeine is formed by *C. arabica* cultures in concentrations as great as in the plant. The productivity of tea cells is significantly lower than that of *C. arabica*. Waller *et al.* (64) first demonstrated the high levels of caffeine of *C. arabica* cultures. Baumann and Frischknecht (24, 25), Baumann (26), and Kurata *et al.* (65, 66, 67) have worked to enhance caffeine biosynthesis in *C. arabica* cell suspension cultures.

From 1995-2002, Waller's laboratory was searching for a bioreactor producing very high levels of caffeine which could be harvested daily for commercial production. When they tried adding yeast at different concentrations to the medium, it occasionally increased caffeine production several hundred times over controls, but the high concentrations were difficult to maintain. Recent work by Waller *et al.* (unpublished) on mixed sterile tissue and cell cultures of *Coffea arabica* and *Paullinia cupana* leaves showed that the medium/callus from coffee/guaraná produced caffeine and theobromine in the ratio 4:1; however, only the medium produced theophylline. There are two possible explanations for the presence of theophylline in the medium: 1) theophylline is bound to chlorogenic acid or other compounds; and 2) it may be able to re-enter the biosynthesis pathway through 3-methyl xanthine to theobromine. Caffeine and theobromine were also produced at higher concentrations in the coffee or guaraná sterile tissue cultures.

7. CATABOLISM OF CAFFEINE

Degradation (catabolism) of caffeine to xanthine has been proven to take place in *Coffea arabica* leaves (4, 19, 33, 40, 68, 69, 70, 71, 72). The purine pathway consists of uric acid, allantoin, allantoic acid, which is degraded to CO_2 and NH_3. In coffee and tea leaves, theobromine is converted almost exclusively to caffeine with little or no release of CO_2, according to tracer experiments which found that theobromine is a precursor of caffeine and not a catabolite of caffeine. Tracer experiments also reveal that theophylline is metabolized rapidly by tea and coffee leaves, whereas labeled caffeine was degraded very slowly. Labeled theophylline was metabolized rapidly by leaves of tea and coffee, indicating that the accumulation of endogenous caffeine in these tissues is a consequence of inadequate 7-demethylase activity in converting caffeine to theophylline.

Leaf caffeine levels in *Coffea eugenoides, C. salvatrix,* and *C. bengalensis* are about 3-7 times smaller than in *C. arabica.* Degradation of labeled caffeine is negligible in leaves of *C. arabica,* and is slow in *C. salvatrix* and *C. bengalensis*. In contrast, labeled caffeine was catabolized rapidly by young leaves of *C. eugenoides*; it proceeded primarily via the caffeine $\rightarrow$ theophylline$\rightarrow$ 3-methylxanthine $\rightarrow$ xanthine$\rightarrow$ uric acid $\rightarrow$ allantoin $\rightarrow$ allantoic acid $\rightarrow$ urea $\rightarrow$ CO_2 and NH_3 pathway (31) (Figure 4). Low amounts of caffeine are found in *C. bengalensis* and *C. salvatrix* as a result of the slow rate of biosynthesis, whereas rapid degradation is responsible for the low endogenous pool in *C. eugenoides*.

The main fate of labeled theophylline incubated with mature and aged tea leaves, and to a lesser extent with young leaves, is conversion of xanthine via 3-methylxanthine and entry into the purine nucleotide catabolic pathway (29). However, if labeled theophylline is fed to young tea leaves, significant amounts of the label show that the biosynthesis of caffeine occurs via a 3-methylxanthine $\rightarrow$ theobromine $\rightarrow$ caffeine pathway. Supporting evidence for the existence of these pathways, which are illustrated in Figure 4, comes from *in vitro* studies of *N*-methyltransferase activity from young tea leaves, which show that xanthine is metabolized to 3-methylxanthine, 3-methyl xanthine is converted to theophylline, and theophylline does not act as a methyl acceptor, and therefore does not undergo direct conversion to caffeine (4, 19, 71).

It follows that there are distinct differences in the purine alkaloid catabolism pathways operating in leaves of *Camellia sinensis* and *Coffea arabica*. Caffeine accumulates in both species, because its catabolism to theophylline undergoes regulation. In young tea leaves there is detectable salvage of 3-methylxanthine and xanthine. There is no evidence for resynthesis of theophylline and of caffeine via theobromine, however, in coffee leaves.

The major pathway via xanthosine in *Theobroma cacao* is almost the same as in young tea leaves (73, 74). The unique accumulation of theobromine observed in cocoa leaves is explained by the very slow conversion of theobromine to caffeine, and because theobromine is directly catabolized by these leaves.

8. CAFFEINE-FREE AND LOW CAFFEINE VARIETIES OF COFFEE

Coffee is one of the most valuable agricultural products to be exported from Central and South America, Africa, and Indonesia. *Coffea arabica* represents about 70 % of the market; *C. robusta* and *C. canephora* make up most of the rest.

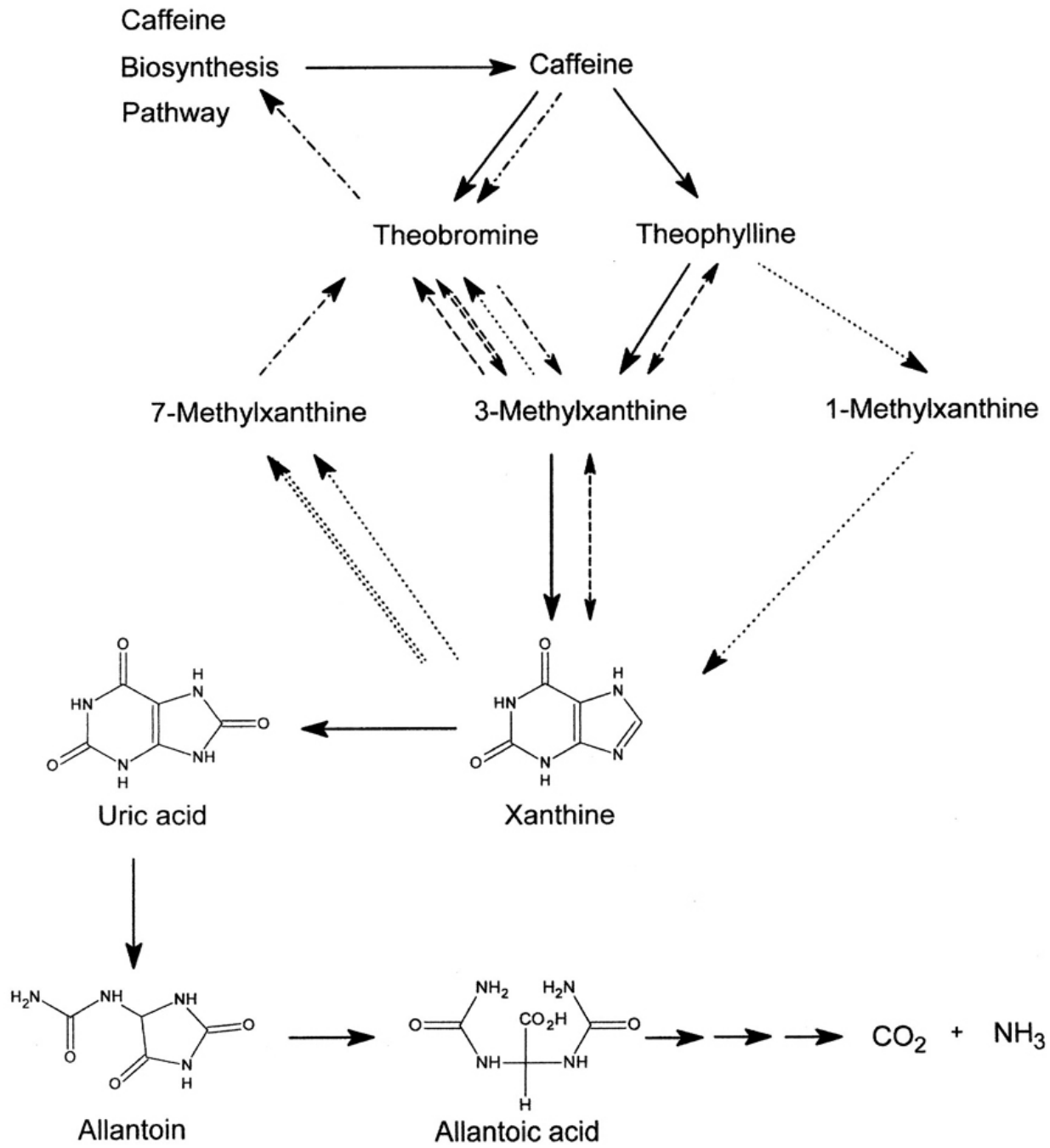

Figure 4. Caffeine catabolism in plants. Solid arrows indicate the main caffeine pathway established originally by Suzuki and Waller (19, 72), and confirmed by several further reports in coffee, tea, and cocoa. Other arrows indicate alternative or minor pathways. Double dotted arrow: *C. arabica* leaves (33); two dots + dashed arrow: *C. dewevrei* leaves and fruits (35); dotted arrow: *C. eugenioides* (31); double dashed arrow: cocoa tea leaves (30); dashed arrow: tea leaves (29); dot + dashed arrow: Kucha tea (14). Reproduced with permission from Ref. 4.

Attempts to breed caffeine-free or low-caffeine plants have not been successful, no matter what varieties of *C. arabica* have been used (4, 75). Usually the flavor, the aroma, or the taste is poor compared to regular coffee produced and used today. Also, the beans are generally much smaller and productivity is lower. Varieties that are caffeine-free or low in caffeine are therefore uncompetitive.

8.1. Patents

At the University of Hawaii, Stiles, Moisyadi, and Neupane hold a series of US patents (77, 78, 79, 80, 81, 82, 83) stemming from research on purified proteins and on DNA sequences that code on hosts transforming coffee plants to reduce the caffeine content.

In Japan, Ashihara, Kato and Mizuno have studied the genes and enzymes responsible for the biosynthesis of caffeine (12, 56). They also hold patents relating to the control of caffeine formation (83, 84, 85, 86, 87). In addition, Sanos's group have been performing research on molecular cloning and expression of *Coffea arabica* genes in the caffeine biosynthetic pathway (caffeine synthetase, theobromine synthase isoforms) in *Escherichia coli* (88, 89, 90, 91).

To understand the caffeine biosynthetic pathway, and to clone the genes related to the production of caffeine, demands detailed knowledge of each *N*-methyltransferase step in the biosynthesis and control of the enzymes and the genes for the production of caffeine. The aim is to control caffeine in coffee and tea. As stated above, genes encoding *N*-methyltransferases for caffeine biosynthesis have been successfully cloned (38, 54, 55) and knockout caffeine coffee plants have been made (57, 58). Suppression of IMPDH gene expression may be an alternative way to produce decaffeinated transgenic tea and coffee plants. Keya *et al.* (92) have shown that inhibition of IMP dehydrogenase by ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) inhibits caffeine and guanine nucleotide biosynthesis in caffeine-forming plants. Use of IMP dehydrogenase-deficient plants as a source of good quality caffeine-deficient tea and coffee plants appears promising.

Using a search strategy to generate decaffeinated coffee, Silvarolla *et al.* (93) found that three *C. arabica* accessions from Ethiopia were almost totally free of caffeine. The seeds of these plants had mean caffeine content 0.76 mg g^{-1} dry weight, whereas the Mundo Novo cultivar of *C. arabica* contains 12 mg g^{-1}. The leaves and other parts of the fruit were also very low in caffeine. Silvarolla and collaborators found that these plants accumulated theobromine (about 6.1 mg g^{-1} dry weight), which is the immediate precursor of caffeine, indicating that these plants might be deficient in caffeine synthase enzyme, which acts on theobromine.

9. ECOLOGICAL ROLE OF ALKALOIDS

Ecological theory predicts that the natural products produced by plants and animals would act to be maximally adaptive for the producers. At least 50 alkaloids have been demonstrated to inhibit the germination or growth of seedlings. These include quinine, cinchonine, ergotamine, harmaline, strychnine, berberine, colchicine, morphine, cocaine, caffeine, coniine, and nicotine, which have been demonstrated to possess allelopathic activity. Many of these compounds have further ecological roles. They are herbivore deterrents, and in many cases they have antibacterial and antifungal properties. These examples of alkaloidal parsimony illustrate the multifunctionality of these compounds and the great adaptiveness of these nitrogen heterocycles (94).

9.1. Herbivory

Plants have evolved strategies against attack by herbivores, microorganisms, viruses, and plant competitors. Plants have open growth and can replace leaves, branches, and roots when wounded. They also have hydrophobic cuticular layers that impede penetration by microbes. Plants possess mechanical weapons (spines, thorns, hooks, trichomes), and produce a wide variety of chemical defense, including indigestible cell walls containing cellulose, lignin, suberin, and callose; glandular and stinging hairs, laticifers and resin ducts, inhibitory or toxic proteins (lectins, protease inhibitors, toxalbumins, eperoxidase, phenolase, ricin, PR (pathogenesis related) proteins such as chitinase, beta-1,3-glucanase, and a wide variety of secondary metabolites (95).

A new view of the biological significance of alkaloids is now proposed. Alkaloids appear to be defensive compounds that act efficiently for their producers against a variety of predatory organisms. Examination of some of the defensive devices employed, *in vivo*, by plant and animal alkaloids clarifies the versatility of these nitrogen-containing compounds as protective agents. By focusing on the alkaloid chemical defense, it also becomes possible to examine the successful offensive strategies of specialist herbivores that exploit host plants fortified with alkaloidal 'forbidden fruits' (94). Although more than 12,000 alkaloids have been described, only about 600 have been analyzed even partly for their biochemical properties, and even fewer for their ecophysiological roles (95).

Since these alkaloids have evolved as deterrents for very different classes of organisms, their production and storage often give rise to mixtures of structurally diverse alkaloids that are stored at specific sites. These accumulations may change in response to factors that optimize reproduction (94).

Some plant species display insecticidal activity as a result of the production of a variety of alkaloids including nicotine, piperine, lupine alkaloids, steroidal alkaloids, ephedrine, berberine, strychnine, gramine, and caffeine. These biologically active alkaloids also act as deterrents to other organisms. Measured by distribution across families, caffeine is the most widely distributed alkaloid, which may be significant because this compound is insecticidal to many species. However, alkaloidal defense against herbivores is not absolute. A number of specialized, unrelated herbivores eat plants containing toxic alkaloids. Some insects have broken through the alkaloid

defenses of selected plant species. In general, plant alkaloids are highly active in deterring a wide range of potential enemies (94).

Crinipellis perniciosa, which is the causal agent of witches' broom disease, attacks actively growing young shoots, flowers and developing fruits of cocoa (*Theobroma cacao*). Infected stem tissue contains significant amounts of caffeine (109±35 mg g^{-1}), approximately seven to eight times more than in healthy stems (14±1 mg g^{-1}). Caffeine production is also stimulated by treatment of young actively growing leaves with salicylic acid, and its synthetic analog benzothiadiazole, which both induce pathogen defense responses in plants. Growth of *C. perniciosa* is also significantly inhibited on caffeine-containing media. These findings suggest that, in cocoa, the caffeine pathway is inducible in young actively growing leaves by pathogen attack, or by wounding and salicylic acid. This may be part of the defense response of this species to herbivory or infection (96).

Baumann *et al.* (97) stated that the fruit of *Paullinia cupana* shows 'bird dispersal syndrome', and that seeds were ingested by toucans and guans. The two aspects of defence and dispersal are reflected in the differential seed alkaloid distribution: the seed kernel and the seed coat accumulate much caffeine, i.e. 4.28 and 1.64%, respectively, whereas the aril, is virtually alkaloid-free, but contains glucose, fructose and sucrose up to almost 70% of aril dry weight. However, cracked seeds release a large fraction of their caffeine, which is believed to be harmful to these birds. Baumann *et al.* (97) also stated that lethal dose of theobromine for birds may be in the range of 1 g per kg body weight. Theobromine is *ca* 10 times less toxic than caffeine in mammals. Applying this factor to birds, the lethal caffeine dose would be in the range of 100 mg per kg body weight. This value corresponds to caffeine toxicity in humans, where the total lethal dose is estimated to vary between 5-100 g.

In some cases, symbiotic relations between plant and alkaloid-producing fungi provide the plants with protection from herbivores. For example, the fungus *Claviceps purpurea* synthesizes quite high concentrations of ergothioneine in the sclerotia; this compound endows grasses with a strong vertebrate toxin that mimics the activity of neurotransmitters such as dopamine, serotonin, and noradrenaline. In this case the fungi appear to utilize nutrients from the grasses while providing them with alkaloidal deterrence (94).

9.2. Allelopathy

The compounds that a plant may add to the environment and that contribute to allelopathy are diverse. Major groups include phenolic acids, glucosinalates, terpenes, and flavonoids. Regardless of the fact that alkaloids form a large group of secondary metabolites, information about their allelopathic activity is rare. This may be because alkaloids have been associated with medicinal uses for centuries, and other possible roles have not been examined (98). However, various studies of the phytotoxicity of alkaloids have been performed; in particular, leachates of coffee leaves and roots, and extracts from soil of coffee plantations, showed a high allelopathic effect (99, 100). Waller and collaborators (101, 102) observed that coffee plants and soils of coffee plantations contain caffeine, methyl esters of myristic through docosanoic acids, furfural, N-phenyl-1-naphthylamine, and N-alkanes.

Young roots of coffee and tea are highly susceptible to caffeine. Since they are close to the soil surface, degeneration, which is known to occur in old coffee plantations, may in some part be due to autotoxicity. The ultimate reasons for caffeine production are complex. It is involved in protection against herbivores, regulation of plant spacing, and suppression of competitive plants. These positive factors presumably override the cumulative negative effects of caffeine, which reduce the life span of trees and bushes (43, 99, 100, 103,104, 105, 106, 107, 108, 109).

The role played by microorganisms in the degradation of caffeine in soils should be included in the study of caffeine as an effective allelopathic compound in coffee and tea plantations. Mazzafera (personal communication) suggests that the soil, acting as a principal source of microorganisms, is a critical factor. Accordingly, Padmanabhan *et al.* (110) sought to develop a field soil biodegradation assay to identify the active microorganisms by analyzing 16S rRNA genes in soil-derived ^{13}C-labeled DNA. They found that caffeine is a slowly metabolized substrate in the soil. By comparing the release of ^{13}C-labeled compounds (glucose, phenol, caffeine, and naphthalene) in differing soil plots and analyzing $^{13}CO_2$ respiration by GC/MS they found that the soil microbe community readily mineralized glucose, phenol, and naphthalene within 24 h, but not caffeine. This study reveals that caffeine can accumulate in the soil of coffee and tea plantations and play an allelopathic role.

To search for practical solutions to the autotoxicity problem in coffee plantations, a study of the allelopathic interactions between arabica coffee and different aromatic plants was performed in a greenhouse, in the laboratory and in the field in Bonn, Germany, and in the coffee Finca Argovia, Chiapas, Mexico (Dr. Jürgen-Pohlan, personal communication). The results indicate that spearmint, oregano and basil are well adapted to grow intercropped with differing coffee cropping systems, using a mechanism that is tolerant of the toxic effects of caffeine. On the other hand, sage significantly inhibits the growth of coffee leaves and branches, and plant height. Spearmint, thyme and oregano clearly increase the growth parameters of young coffee plants. Potential detoxification of caffeine through sage, thyme and rosemary may diminish toxic accumulation of the allelochemical in old coffee plantations, and increase coffee production. Laboratory experiments on caffeine uptake indicate that all aromatic species absorb caffeine, and that it accumulated mainly in the roots.

9.2.1. Mechanisms of action of caffeine and other purine alkaloids in plants

Several experiments performed by Waller's group and Anaya's group showed that, when coffee seeds

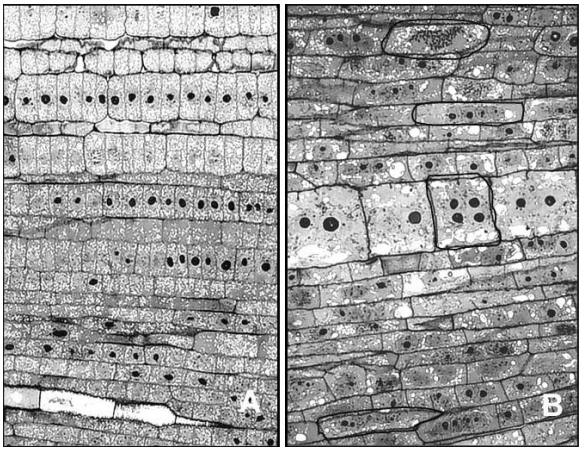

Figure 5. Micrographs of stele tissues and cells of maize roots, 72 h after germination. A. Control; B. caffeine treated tissues (x 40) Reproduced with permission from Ref. 104.

were allowed to germinate in aqueous solutions of caffeine at various concentrations, elongation of the hypocotyls was reduced and growth of rootlets was almost completely inhibited by 10 mM caffeine (99, 105, 106). Friedman and Waller (105) observed that mitosis and cell plate formation were also inhibited. The root tips were darkened, and 4-5 days after the treatment they had deteriorated. Similar though milder inhibition was caused by theophylline. In natural conditions, embryos of germinating coffee seeds must be able to avoid autotoxic hazards from their endogenous caffeine. Germinating coffee seeds contain an average of 40 mM caffeine. However, mitosis in root tips is arrested by 10 mM caffeine *in vitro*. A likely resolution is that caffeine in coffee seeds is stored in the endosperm. The dormant embryo is nearly free of the alkaloid. In the first stages of germination there is no cell division in the root tip. Cell division begins only after the tip is pushed outside (1-3 mm away from the caffeine-rich endosperm) as a result of cell expansion and elongation of the hypocotyl. During this stage the cotyledons of the embryo are about 2 mm in diameter, and mitosis proceeds until the third week of germination. At the end of this stage, caffeine vanishes from the endosperm, accumulating in the cotyledons once mitosis is arrested. At the final stage of germination, most of the caffeine in seedlings is in cotyledons (85%) and in the hypocotyl (13%), with only a small amount in the root.

Friedman and Waller (106) proposed that caffeine may operate by incorporation into nucleic acid chains (DNA and RNA), preventing normal cell division. Wink and Twardowski (111) stated that alkaloids interact with DNA by intercalation with nucleic acids by ionic bonding of the positively charged heterocyclic nitrogen of the alkaloids to the negatively charged phosphate groups of nucleic acids. Replication or translation (protein biosynthesis) might therefore be affected by these molecules, since they are represent potential targets and are basic to all living systems.

Anaya *et al.* (104) studied the effects of caffeine on root growth and root ultra structure of maize. They reported that the LC_{50} on the root growth of maize was 500 ppm. Under optical microscopy, the root apical meristem of maize after 72 h of treatment with a 500 ppm caffeine solution showed a zone of compressed cells between the cap and the meristem. Some of these cells had irregular shapes, two nuclei, diffuse nuclei, and also two or more nucleoli (Figure 5).

It has also been reported that caffeine inhibited cell plate formation during telophase in coffee plants, *Tradescantia*, and *Allium cepa*, and inhibits cytokinesis in wheat roots (112, 113, 114). These reports accord with the findings of Anaya *et al.* (104) that extend and support studies of the fragmoplast, cell plates, and of cytokinesis inhibition.

Other studies have described the effect of caffeine on the various phases of the cell cycle (G_1, S, G2, M) [footnote], specifically in replication and G_2 checkpoints. Pelayo *et al.* (115) found that, in bulbs of *Allium cepa*, 5 mM caffeine selectively abrogated the G_2 block produced by the checkpoint that controls post-replication DNA repair, since it promotes the entry of cells into an aberrant mitosis. The same group also showed that 5 mM caffeine increases the effect of gamma-irradiation, and pointed out that this potentiation effect of caffeine on induced chromosomal damage may be due not only to caffeine-induced cancellation of the G_2 checkpoint, but also to a direct effect of methylxanthine on the processing of DNA damage (116).

10. PERSPECTIVE

Caffeine-containing products have been consumed for hundreds of years for their delightful flavor and stimulating effects. The most common caffeine-rich beverages are coffee, tea, maté, guaraná and cocoa. By far the two most commonly consumed beverages in the world today are coffee and tea, which are produced by hot water extraction of the roasted beans (coffee) or leaf (tea).

The recent extraordinary technological advances in purine alkaloid research have taken place worldwide. The laboratories involved continue to investigate in further detail the complex evolutionary, ecological, physiological, and metabolic processes occurring in caffeine-containing plants. The use of modern and advanced techniques in genetics and cloning, biochemistry, molecular biology, and new bioassays, can reasonably be called a revolution, and will continue to be developed in the future.

In this review we have highlighted the importance of allelopathic interactions and the role of purine alkaloids such as caffeine, theobromine, and theophylline which are probably important in coffee and tea autotoxicity. Allelochemical interactions between plants, as part of interference, are now acknowledged to be a key factor in the patterning of vegetation and weed growth, and in crop yield, in agricultural systems. Secondary compounds with allelochemical potential make up the spectrum of known allelopathic chemicals that are involved in many metabolic and ecological processes.

The mechanisms of action of these compounds are an important aspect of allelopathy, in which biochemical approaches are employed. However, the adverse effects of allelochemicals arising from purine alkaloid producing plants are not well understood. Consequently, there is a need to evaluate the production of coffee, tea, maté, and guaraná plantations to determine the effects of previously planted crops and residues. We anticipate that the tools of biotechnology will increasingly be applied to coffee and tea plantations without any effect on the quality of the resulting beverage.

11. ACKNOWLEDGEMENTS

We are very grateful to Drs. Hiroshi Ashihara and Paulo Mazzafera for reading and commenting critically on this review article. We thank Cecilia Padierna Mota for preparing drawings, and the Oklahoma Agricultural Experiment for their encouragement.

12. REFERENCES

1. M F Roberts, and M. Wink: Introduction. In: Alkaloids: Biochemistry, Ecology, and Medicinal Applications. Eds: Roberts, M F. and Wink M. Plenum Press. NY and London, 1-7 (1998)

2. S W. Pelletier: The nature and definition of an alkaloid. In: Alkaloids: Chemical and Biological Perspectives. Vol. 1. Ed: Pelletier, S.W, Wiley, NY. 1-31 (1983)

3. Rosemeyer H: The chemodiversity of purine as a constituent of natural products. *Chem and Biodivers* 1, 361-401 (2004)

4. Mazzafera P: Catabolism of caffeine in plants and microorganisms. *Front Biosc* 9, 1348-1359 (2004)

5. Mösli Waldhauser S S and T W Baumann: Compartmentation of caffeine and related purine alkaloids depends exclusively on the physical chemistry of their vacuolar complex formation with chlorogenic acids. *Phytochemistry* 42, 985-996 (1996)

6. R R Scheline: Handbook of Mammalian Metabolism of Plant Compounds. CRC Press, Boca Raton. (1991)

7. Barr H M and P Streissguth: Caffeine use during pregnancy and child outcome: a seven year protective study. *Neurotox Teratol* 13, 441-448 (1991)

8. Kimmel C A, G L Kimmel, C White, T E Grasto, J F Young and G J Nelson: Blood flow changes and conceptual development in pregnant rats in response to caffeine. *Fund Appl Toxicol* 4, 240-247 (1984)

9. Concannon I T, J M Braughler and M D Chechter: Pre and post natal effects of caffeine on brain biogenic amines, cyclic nucleotides and behaviour in developing rats. *J Pharmacol Exp Ther* 226, 673-679 (1983)

10. Huges R N and I J Beveridge: Behavioural effects of exposure to caffeine during gestation, lactation or both. *Neurotoxicol Teratol* 13, 641-647 (1991)

11. Waldvogel S R: Caffein-ein wirkstoff mit überraschungen. *Angewandte Chemie* 115, 624-625 (2003)

12. Ashihara H and T Suzuki: Distribution and biosynthesis of caffeine in plants. *Front Biosci* 9, 1864-1876 (2004)

13. Nagata T and S Sakai: Purine base pattern of *Camellia irrawadiensis. Phytochemistry* 24, 2271-2272 (1985)

14. Zheng X Q, C X Yeb, M Kato, A Crozier and H Ashihara: Theacrine (1,3,7,9-tetramethyluric acid) synthesis in leaves of a Chinese tea, kucha (*Camellia assamica* var. *kucha*). *Phytochemistry* 60, 129-134 (2002)

15. Weckerle C S, M A Stutz and T W Baumann: Purine alkaloids in *Paullinia*. *Phytochemistry* 64, 735-42 (2003)

16. Kretschmar J A and T W Baumann: Caffeine in *Citrus* flowers. *Phytochem* 52, 19-23 (1999)

17. Weevers T: The function of Xanthine Derivatives in Plant Materials, Archives Neerlandaises, Series III, 5, 111-195 (1930)

18. Waller G. R: Introduction- Reality and Future of Allelopathy. In: Allelopathy: Chemistry and Mode of Action of Allelochemicals. Eds: Macias F A, Galindo J M, Molinillo J M G., and Cutler H G.. CRC Press, Boca Raton, FL, USA.1-17 (2004)

19. Suzuki T and G. R Waller: Biodegradation of caffeine: Formation of theophylline and theobromine from caffeine in mature *Coffea Arabica* fruits. *J Sci Food Agric* 35, 66-70 (1984)

20. Roberts M F and G. R Waller: *N*-Methyltransferases and 7-Methyl-N-9'-nucleoside hydrolase activity in *Coffea arabica* and the biosynthesis of caffeine. *Phytochemistry* 18, 451-455 (1979)

21. Suzuki T and G. R Waller: Metabolism of caffeine in sterile tissue cultures of *Coffea arabica*. *Proc. 5th Int. Plant Tissue Cell Culture* 385-386 (1982)

22. Zheng X Q and H Ashihara: Distribution, biosynthesis and function of purine and pyridine alkaloids in *Coffea arabica* seedlings. *Plant Sci* 166, 807-813 (2004)

23. G. R Waller, J Friedman, C H Chou, T Suzuki and N Friedman: Hazards, benefits, metabolism and translocation of caffeine in *Coffea arabica* L. plants and surrounding soil. In: Proceedings of the Seminar on Allelochemicals and Pheromones, June 21-26, 1982, Monograph 5, Institute of Botany, Academia. Sinica, Taipei, ROC, 239-260 (1983)

24. Baumann T W and P M Frischknecht: Biosynthesis and biodegradation of purine alkaloids in tissue cultures. *Proc.*

5th Int. Congress. Plant Tissue and Cell Culture, 365-366 (1982)

25. T W Baumann and P M Frischknecht: Caffeine: production by plant (*Coffea* spp.) cell cultures. In: Medicinal and Aromatic Plants I. Ed: Bajaj Y P S. Springer Verlag, Berlin, 264-281 (1988)

26. Baumann T W: Caffeine. *Bot Helv* 106, 127-158 (1996)

27. Ashihara H, A M Monteiro, F M Gilles and A Crozier: Biosynthesis of caffeine in leaves of coffee. *Plant Physiol* 111, 747-753 (1996)

28. Ashihara H, A M Monteiro, T Moritz, F M Gilles and A Crozier: Catabolism of caffeine and related purine alkaloids in leaves of *Coffea arabica L. Planta* 198, 334-339 (1996)

29. Ashihara H, F M Gilles and A Crozier: Metabolism of caffeine and related purine alkaloids in leaves of tea (*Camellia sinensis L.). Plant Cell Physiol* 38, 413-419 (1997)

30. Ashihara H, M Kato and C X- Ye: Biosynthesis and metabolism of purine alkaloids in leaves of cocoa tea (*Camellia ptilophylla*). *J Plant Res* 101, 599-604 (1998)

31. Ashihara H and A Crozier: Biosynthesis and catabolism of caffeine in low-caffeine containing species of *Coffea. J Agric Food Chem* 47, 3425-3431 (1999)

32. Ashihara H and A Crozier: Biosynthesis and metabolism of caffeine and purine alkaloids in plants. *Adv Bot Res* 30, 117-205 (1999)

33. Ashihara H and A Crozier: Caffeine: a well known but little mentioned compound in plant science. *Trends Plant Sci* 6, 407-413 (2001)

34. Mazzafera P, A Crozier and A C Magalhaes: Caffeine metabolism in *Coffea arabica* and other species of coffee. *Phytochemistry* 30, 3913-1916 (1991)

35. Mazzafera P, A Crozier and G Sandberg: Studies on the metabolic control of caffeine turnover in developing endosperms and leaves of *Coffea arabica* and *Coffea dewevrei. J Agr Food Chem* 42, 1423 (1994)

36. Mazzafera P: 7-Methylxanthine is not involved in caffeine catabolism in *Coffea dewevrei*. *J Agr Food Chem* 41, 1541-1543 (1993)

37. Koshiishi C, A Kato, S Yama, A Crozier and H Ashihara: A new caffeine biosynthetic pathway in tea leaves: utilization of adenosine released from the *S*-adenosyl-L-methionine cycle*. FEBS Lett* 499, 50-54 (2001)

38. Kato M, K Mizuno, A Crozier, T Fujimura and H Ashihara: Caffeine synthase gene from tea leaves. *Nature* 406, 956-957 (2000)

39. Ogawa M, Y. Herai, T Koizumi, T Kuzano and H Sano: 7-Methylxanthine methyltransferase of coffee plants. Gene isolation and enzymatic properties*. J Biol Chem* 276, 8213-8218 (2001)

40. Suzuki T, H Ashihara and G R Waller: Purine and purine alkaloid metabolism in *Camellia* and *Coffea* plants. *Phytochemistry* 31, 2575–2584 (1992)

41. Ito E, A Crozier and H Ashihara: Theophylline metabolism in higher plants. *Biochim Biophys Acta G S* 1336**,** 323-330 (1997)

42. Ito E and H Ashihara: Contribution of purine nucleotide biosynthesis *de novo* to the formation of caffeine in young tea (*Camellia sinensis*) leaves. *J Plant Physiol* 154, 145-151 (1999)

43. G. R Waller, H Ashihara, M Kato, T W Baumann, A Crozier and T Suzuki: Pathways Involved in the Metabolism of Caffeine by *Coffea* and *Camellia* Plants. In: Caffeinated Beverages: Health Benefits, Physiological Effects, and Chemistry Eds: Parliament T H, Ho C.-T and Schieberle P. ACS Series 754, American Chemical Society, Washington, D. C., 9-19 (2000)

44. Schulthess B H, P Morath and T W Baumann: Caffeine biosynthesis starts with the metabolically channeled formation of 7-methyl-XMP: a new hypothesis. *Phytochemistry* 41, 169-175 (1996)

45. Negishi, O, T Osawa and H Imagawa: Methylation of xanthosine by tea-leaf extracts and caffeine biosynthesis. *Agr Biol Chem* 49, 887-890 (1985)

46. Kato M, T Kanehara, H Shimizu, T Suzuki, F M Gillies, A Crozier and H Ashihara: Caffeine biosynthesis in young leaves of *Camellia sinensis* L.: *in vitro* studies on *N*-methyltransferase activity involved in the conversion of xanthosine to caffeine. *Physiol Plantarum* 98, 629-636 (1996)

47. Mosli Waldhauser S S, F M Gilles, A Crozier and T W Baumann: Separation of the *N-7* methyltransferase, the key enzyme in caffeine biosynthesis. *Phytochemistry* 45, 1407-1414 (1997)

48. Moisyadi S, K R Neupane and J I Stiles: Cloning and characterization of a cDNA encoding xanthosine N7 methyltransferase from coffee (*Coffea arabica*). *Acta Horticulturae* 461, 367-377 (1998)

49. S Moisyadi and J I Stiles: Cloning and characterization of xanthosine-*N7*-methyltransferase, the first enzyme of the caffeine biosynthetic pathway. In: Proceedings of the 18th International Scientific Colloquium on Coffee, 327-331, Association Scientifique Internationale du Café, Paris, France (1999)

50. Negishi O, T Osawa and H Imagawa: *N*-methylnucleosidase from tea leaves. *Agrl Biol Chem* 52, 169-175 (1988)

51. Suzuki T and E Takahashi: Metabolism of xanthine and hypoxanthine in the tea plant (*Thea sinensis* L.). *Biochem J* 146, 79-85 (1975)

52. Suzuki T and E Takahashi: Biosynthesis of caffeine in tea-leaf extracts: enzyme formation of theobromine from 7-methylxanthine and of caffeine from theobromine. *Biochem J* 146, 87-96 (1975)

53. Kato M, K Mizuno, T Fujimura, M Iwama, M Irie, A Crozier and H Ashihara: Purification and characterization of caffeine synthase from tea leaves. *Plant Physiol* 120, 579-586 (1999)

54. Mizuno K, A Okuda, M Kato, N Yoneyama, H Tanaka, H Ashihara and T Fujimura: Isolation of a new dual-functional caffeine synthase gene encoding an enzyme for the conversion of 7-methylxanthine to caffeine from coffee (*C. arabica*). *FEBS Lett* 534, 75-81 (2003)

55. Mizuno K, M Kato, F Irino, N Yoneyama, T Fujimura and H Ashihara: The first committed step reaction of caffeine biosynthesis: 7-methylxanthosine synthase is closely homologous to caffeine synthases in coffee (*Coffea arabica L.*). *FEBS Lett* 547, 56-60 (2003)

56. Kato M and K Mizuno: Caffeine synthase and related methyltransferases in plants. *Front Biosci* 9, 1833-1842 (2004)

57. Ogita S, H Uefuji, Y Yamaguchi, N Koizumi and H Sano: Producing decaffeinated coffee plants. *Nature* 423, 823 (2003)

58. Ogita S, H Uefuji, M Morimoto and H Sano: Application of RNAi to confirm theobromine as the major intermediate for caffeine biosynthesis in coffee plants with potential for construction of decaffeinated varieties. *Plant Mol Biol* 54, 931-941 (2004)

59. Uefuji H, S Ogita, Y Yamaguchi, N Koizumi and H. Sano: Molecular cloning and functional characterization of three distinct *N*-methyltransferases involved in the caffeine biosynthetic pathway in coffee plants. *Plant Physiol* 132, 372-380 (2003)

60. Ogutuga D B A and D H Northcote: Caffeine formation in tea callus tissue. *J Exp Bot* 21 (67), 258-273 (1970)

61. Ogutuga D B A and D H Northcote: Biosynthesis of caffeine in tea callus tissue. *Biochem J* 117, 715-720 (1970)

62. Tsushida T and Y Doi: Nippon Nogeikagaku Kaishi, 58: 1131-1133 (1984) (in Japanese with English summary)

63. Keller H, H Wanner and T W Baumann: Caffeine synthesis in fruits and tissue culture of *Coffea arabica*. *Planta* 108, 339-350 (1972)

64. Waller G R, C D McVean and T Suzuki: High production of caffeine and related enzyme activities in callus cultures of *Coffea arabica* L. *Plant Cell Rep* 2, 109-112 (1983)

65. Kurata H, S Matsumura and S Furusaki: Light irradiation causes physiological and metabolic changes for purine alkaloid production by a *Coffea arabica* cell suspension culture. *Plant Sci* 123, 197-203 (1997)

66. Kurata H, T Achioku, N Okuda, S Furusaki: Intermittent light irradiation with a second-scale interval enhances caffeine production by *Coffea arabica* cells. *Biotechnol Progr* 14, 797-799 (1998)

67. Kurata H, T Achioku, S Furusaki: The light/dark cycle operation with an hour-scale period enhances caffeine production by *Coffea arabica* cells. *Enzyme Microb Technol* 23, 518-523 (1998)

68. Kalberer P: Breakdown of caffeine in the leaves of *Coffea arabica* L. *Ber. Schweiz. Bot. Ges* 74, 62-107 (1964)

69. Kalberer. P: Breakdown of caffeine in the leaves of *Coffea Arabica. Nature* 205, 597-598 (1965) 71.

70. Spedding D J and A T Wilson: Caffeine metabolism. *Nature* 204, 73 (1964)

71. Suzuki T and G R Waller: Biosynthesis and biodegradation of theobromine and theophylline in *Coffea arabica* fruits. *J Agr Food Chem* 32, 845-848 (1984)

72. T Suzuki and G R Waller: Metabolism and analysis of caffeine and other methylxanthines in coffee, tea, cola, guaraná and cocoa. In*:* Modern Methods of Plant Analysis, New Series, Vol. 8, Analysis of Nonalcoholic Beverages. Eds: Linskens, H. F. and Jackson, J. F. Springer-Verlag, Berlin, pp. 184–220 (1988)

73. Koyama Y, Y Tomado, M Kato and H Ashihara: Metabolism of purine bases, nucleosides and alkaloids in theobromine forming *Theobroma cacao* leaves. *Plant Physiol Biochem* 41, 977-984 (2003)

74. Zheng X Q, Y Koyama, C Nagai and H Ashihara: Biosynthesis, accumulation and degradation of theobromine in developing *Theobroma* cacao fruits. *J Plant Physiol* 161, 363–369 (2004)

75. Barros R S, M Moacyr and B R Alemar: Purine alkaloids in *Coffea. J Coffee Res* 24, 55-86 (1994)

76. Stiles J I, I Moisyadi and K R Neupane: Purified proteins, recombinant DNA sequences and processes for producing caffeine-free beverages. Int. Patent Public. No. WO 97/35960, October 1997 (1997)

77. Stiles J I, I Moisyadi and K R Neupane: Purified proteins, recombinant DNA sequences and processes for

producing caffeine-free beverages. Int. Patent Public. No. WO 98/42848, October 1998 (1998)

78. Stiles J I, I Moisyadi and K R Neupane: Purified proteins, recombinant DNA sequences and processes for controlling the ripening of coffee plant. United States Patent number 5,874,269. February 23, 1999 (1999)

79. Stiles J I, I Moisyadi and K R Neupane: Purified proteins, recombinant DNA sequences and processes for producing caffeine-free beverages. United States Patent 6,075,184. June 13, 2000 (2000)

80. Stiles J I, I Moisyadi and K R Neupane: Purified proteins, recombinant DNA sequences and processes for producing caffeine-free beverages. United States Patent 6,348,641 B1. February 19, 2002 (2002)

81. Stiles J I, I Moisyadi and K R Neupane: Purified proteins, recombinant DNA sequences and processes for controlling the ripening of coffee plant. United States Patent 6,448,474 B1. September 10, 2002 (2002)

82. Stiles J I, I Moisyadi and K R Neupane: Purified proteins, recombinant DNA sequences and processes for controlling the ripening of coffee plant. United States Patent 6,727,406 B2. April 27, 2004 (2004)

83. Mizuno M, H Ashihara, K Mizuno and T Fujimura: Protein and cDNA sequences of a novel *Camellia sinensis* N-methyl transferase involved in caffeine biosynthesis and uses thereof. Japan Patent Number JP2001037490. May 23, 2000 (2000)

84. Mizuno M, A Ashihara, K Mizuno and T Fujimura: cDNA and protein sequences of coffee and tea *N*-methyltransferase and their uses for caffeine synthesis. Japan Patent Number 2002085072. March 26, 2002 (2002)

85. Mizuno K, H Kanaka, T Fujimora, M Mizuno and A Ashihara: cDNA cloning of theobromine synthase from coffee (*Coffea arabica* L.). Japan Patent Number JP2003088372. March 25, 2003 (2003)

86. Mizuno M, H Ashihara and K Mizuno: Caffeine synthase and gene for coding the synthase. Japan Patent Number JP2003088373. March 25, 2003 (2003)

87. Mizuno K, M Mizuno, A Okuda, T Fujimora and A Ashihara: Caffeine synthases from coffee (*Coffea arabica* L.) and use for caffeine biosynthesis. Japan Patent Number JP2004105075. April 8, 2004 (2004)

88. Sano H, T Kusano and N Koizumi: cDNA and protein sequences of *Coffea arabica* theobromine synthase isoforms and their use for caffeine biosynthesis. Japan Patent Number 2002112785. April 16, 2002 (2002)

89. Uefuji H, H Sano, N Koizumi and A Shinmyo: Caffeine synthetase and use thereof. Japan Patent Number WO 03/087376 A1. October 23, 2003 (2003)

90. Uefuji H, H Sano, N Kolzumi, A Simio: Molecular cloning and expression of the *Coffea arabica* genes in the caffeine biosynthetic pathway in *Escherichia coli.* Japan Patent Number JP2004049182. February 19, 2004; US Patent Number US2004128709. July 1, 2004 (2004)

91. Kamifuji H, H Sano, N Koizumi and A Niina: Caffeine synthetase and its use. Japan Patent Number JP2003304879. Oct. 28, 2003 (2003)

92. Keya C A, A Crozier and H Ashihara: Inhibition of caffeine biosynthesis in tea (*Camellia sinensis*) and coffee (*Coffea arabica*) plants by ribavirin. *FEBS Lett* 554, 473-477 (2003)

93. Silvarolla M B, P Mazzafera, P and L C Fazuoli: A naturally decaffeinated arabica coffee. *Nature* 429, 826 (2004)

94. M S Blum: The importance of alkaloidal functions. In: Allelopathy: Chemistry and Mode of Action of Allelochemicals. In: Eds: Macías F A, Galindo J C G, Molinillo J M G, Cutler H G. CRC Press, Boca Raton, FL. 163-181 (2004)

95. M Wink: Chemical Ecology of Alkaloids. In: Alkaloids: Biochemistry, Ecology, and Medicinal Applications. Eds: Roberts M F and Wink M. Plenum Press. New York and London. 265-300 (1998)

96. Aneja M and T Gianfagna: Induction and accumulation of caffeine in young, actively growing leaves of cocoa (*Theobroma cacao* L.) by wounding or infection with *Crinipellis perniciosa*. *Physiol Mol Plant P* 59, 13-16 (2001)

97. Baumann T W, B H Schulthess and K Hanni: Guaraná (*Paullinia cupana*) rewards seed dispersers without intoxicating them by caffeine. *Phytochem* 39, 1063-1070 (1995)

98. J V Lovett and A H C Hoult: Allelopathy in Plants. In: Alkaloids: Biochemistry, Ecology, and Medicinal Applications. Eds. Roberts M F and Wink M, Plenum Press, NY London, 337- 347 (1998)

99. A L Anaya, L Ramos, J G Hernandez and R Cruz-Ortega: Allelopathy in Mexico. In: Allelochemicals: Role in Agriculture and Forestry. Ed: Waller G R, *ACS Series* 330, *American Chemical Society*, Washington, D. C. 89-101 (1987)

100. A L Anaya, R Cruz-Ortega and V Nava-Rodriguez: Impact of allelopathy in the traditional management of agroecosystems in Mexico. In: Allelopathy: Basic and applied aspects. Eds: Rizvi S J H, Rizvi V, Chapman and Hall, London. 271-301 (1992)

101. Waller G R, D Kumari, J Friedman, N Friedman and Ch-H Chou: Caffeine Autotoxicity in *Coffea arabica* L. In: The Science of Allelopathy. Eds: Putnam A R and Tang Ch-Sh. John Wiley & Sons. NY. 243-269 (1986)

102. Waller G R, A L Anaya, L Sagrero-Nieves, A B Celis Ochoa Cordero, D Kumari, J Friedman, N Friedman and Ch-H Chou: A problem in coffee plantations: autotoxicity of caffeine and other compounds. ASIC. Proceedings of 13e Colloque. Paipa, Colombia: 363-371 (1989)

103. Anaya A L: Allelopathy as a Tool in the Management of Biotic Resources in Agroecosystems. *Crit Rev Plant Sci* 18, 697-739 (1999)

104. A L Anaya, G R Waller, P Okinda-Owuor, J Friedman, C H Chou, T Suzuki, J F Arroyo-Estrada and R Cruz-Ortega: The Role of Caffeine in the Production Decline due to Autotoxicity in Coffee and Tea Plantations. In: Allelopathy: From Molecules to Ecosystems. Eds: Reigosa M and Pedrol N. Science Publishers Inc. Enfield, USA 71-91 (2002)

105. Friedman J and G R Waller: Caffeine hazards and their prevention in germinating seeds of coffee (*Coffea arabica*). *J Chem Ecol* 9, 1099-1106 (1983)

106. Friedman J and G R Waller: Seeds as allelopathic agents. *J Chem Ecol* 9, 1107-1117 (1983)

107. Friedman J and G R Waller: Allelopathy and autotoxicity. *Trends Biochem Sci* 10, 47-50 (1985)

108. G R Waller: Allelochemical Action of Some Natural Products. In**:** Phytochemical Ecology: Allelochemicals, Mycotoxins, and Insect Pheromones and Allomones. Eds: Chou, Ch H and Waller G R. Inst Bot, *Academia Sinica, Monograph Series* No.9, Taipei, ROC. 129-154 (1989)

109. Waller G R and F A Einhellig: Overview of Allelopathy in Agriculture, Forestry, and Ecology. In: Biodiversity and Allelopathy: From Organisms to Ecosystems in the Pacific**.** Eds: Chou Ch H, Waller G R and Reinhardt C. Academia Sinica, Taipei, ROC. 221-245 (1999)

110. Padmanabhan P, S Padmanabhan, C DeRito, A Gray, D Gannon, J R Snape, C S Tsai, W Park, C Jeon and E L Madsen: Respiration of ^{13}C-labeled substrates added to soil in the field and subsequent 16S rRNA gene analysis of ^{13}C-labeled soil DNA. *Appl Environ Microb* 69, 1614-1622 (2003)

111. Wink M and T Twardowski: Allelochemical Properties of Alkaloids: Effects on Plants, Bacteria and Protein Biosynthesis. In: Allelopathy: Basic and Applied Aspects. Eds: Rizvi S J H and Rizvi V. Chapman and Hall. NY 129-150 (1992)

112. Valster A H and P K Hepler: Caffeine inhibition of cytokinesis: Effect on the phragmoplast cytoskeleton in living *Tradescantia* stamen hair cell. *Protoplasma* 196, 155-166 (1997)

113. Thomas J, Q Chen and N Howes: Chromosome doubling of haploids of common wheat with caffeine. *Genome V*. 40, 552-558 (1997)

114. Gimenez-Abian M I, L Utrila, J L Canovas, G Jimenez-Martinez, M H Navarrete and C de la Torre: The positional control of mitosis and cytokinesis in higher-plant cells. *Planta* 204**,** 37-43 (1998)

115. Pelayo H R, P Lastresand C De la Torre: Replication and G_2 checkpoints: their response to caffeine. *Planta* 212, 444-453 (2001)

116. Pincheira J, J F Lopez-Saez, P Carrera, M H Navarrete and C De la Torre: Effect of caffeine on *in vivo* processing of alkylated bases in proliferating plant cells. *Cell Biol Int* 27, 837-843 (2003)

Footnote: G1: the longest phase during which the cells are preparing for DNA replication; **S:** the only period in the cell cycle during which DNA is replicated; **G2:** a short gap before mitosis; **M:** mitosis (separation of the daughter chromosomes) and cell division.

Key Words: Allelopathy, Biosynthesis, Caffeine, *Camellia,* Catabolism, Cell Cultures, *Citrus, Coffea, Cola,* Ecological role*,* Herbivory, *Ilex,* Metabolism, Modes of Action, *Paullinia,* Patents, Purine Alkaloids, Theobromine, Theophylline, Review

Send correspondence to: Dr Ana Luisa Anaya, Laboratorio de Alelopatia, Instituto de Ecologia, Universidad Nacional Autonoma de Mexico, Circuito Exterior, Ciudad Universitaria, 04510, Mexico, D.F., Tel: 52-55-5622-9032, Fax 52-55-5616-1976, E-mail: alanaya@miranda.ecologia.unam.mx

http://www.bioscience.org/current/vol11.htm