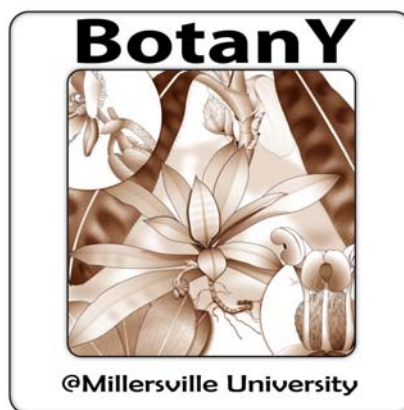


## Topic 09



# Secondary Metabolites

Raven Chap. 2 (pp. 30-35)

Bring pre-washed white t-shirt to lab this week!

## I. Plant Secondary Metabolites

### A. Definitions

#### 1) Secondary Metabolism-

#### 1a) Metabolite-

## I. Plant Secondary Metabolites

### B. Examples

Compound	Example Source	Human Use
<b>ALKALOIDS</b>		
Codeine	Opium poppy	Narcotic pain relief; cough suppressant
Nicotine	Tobacco	Narcotic; stimulant
Quinine	Quinine tree	Used to treat malaria; tonic
<b>Cocaine</b>	Coca	Narcotic, tea, anesthetic, stimulant
<b>PHENOLICS</b>		
Lignin	Woody plants	Hardwood furniture & baseball bats
<b>Tannin</b>	Leaves, bark, acorns	Leather tanning, astringents
Salicin	Willows	Aspirin precursor
Tetrahydrocannabinol	Cannabis	Treatment for glaucoma & nausea
<b>TERPENOIDS</b>		
Camphor	Camphor tree	Component of medicinal oils, disinfectants
Menthol	Mints & eucalyptus	Strong aroma; cough medicines

## I. Plant Secondary Metabolites

### C. Ecology

Open access, freely available online PLOS BIOLOGY

## Nicotine's Defensive Function in Nature

Anke Steppuhn, Klaus Gase, Bernd Krock, Rayko Halitschke, Ian T. Baldwin\*

Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Jena, Germany

Plants produce metabolites that directly decrease herbivore performance, and as a consequence, herbivores are selected for resistance to these metabolites. To determine whether these metabolites actually function as defenses requires measuring the performance of plants that are altered only in the production of a certain metabolite. To date, the defensive value of most plant resistance traits has not been demonstrated in nature. We transformed native tobacco (*Nicotiana attenuata*) with a consensus fragment of its two *putrescine N-methyl transferase (pmt)* genes in either antisense or inverted-repeat (IR*pmt*) orientations. Only the latter reduced (by greater than 95%) constitutive and inducible nicotine. With D<sub>4</sub>-nicotinic acid (NA), we demonstrate that silencing *pmt* inhibits nicotine production, while the excess NA dimerizes to form anatabine. Larvae of the nicotine-adapted herbivore *Manduca sexta* (tobacco hornworm) grew faster and, like the beetle *Diabrotica undecimpunctata*, preferred IR*pmt* plants in choice tests. When planted in their native habitat, IR*pmt* plants were attacked more frequently and, compared to wild-type plants, lost 3-fold more leaf area from a variety of native herbivores, of which the beet armyworm, *Spodoptera exigua*, and *Trimerotropis* spp. grasshoppers caused the most damage. These results provide strong evidence that nicotine functions as an efficient defense in nature and highlights the value of transgenic techniques for ecological research.

Citation: Steppuhn A, Gase K, Krock B, Halitschke R, Baldwin IT (2004) Nicotine's defensive function in nature. PLoS Biol 2(8): e117.

Steppuhn et al. 2004. *PLoS Biology* 2: 1074-1080.

## I. Plant Secondary Metabolites

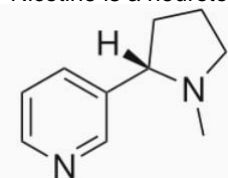
### C. Ecology

Nicotine negatively affects function of herbivores.



Tobacco (*Nicotiana tabacum*)

Nicotine is a neurotoxin.



Nicotine is made in roots and transported to shoots via xylem.



Tobacco (*Nicotiana tabacum*)

Most potential herbivores cannot deal with nicotine.



The tobacco hornworm (a moth larva) can sequester and secrete nicotine, with some energetic cost.

## An Ecologically Motivated Analysis of Plant-Herbivore Interactions in Native Tobacco<sup>1</sup>

Ian T. Baldwin\*


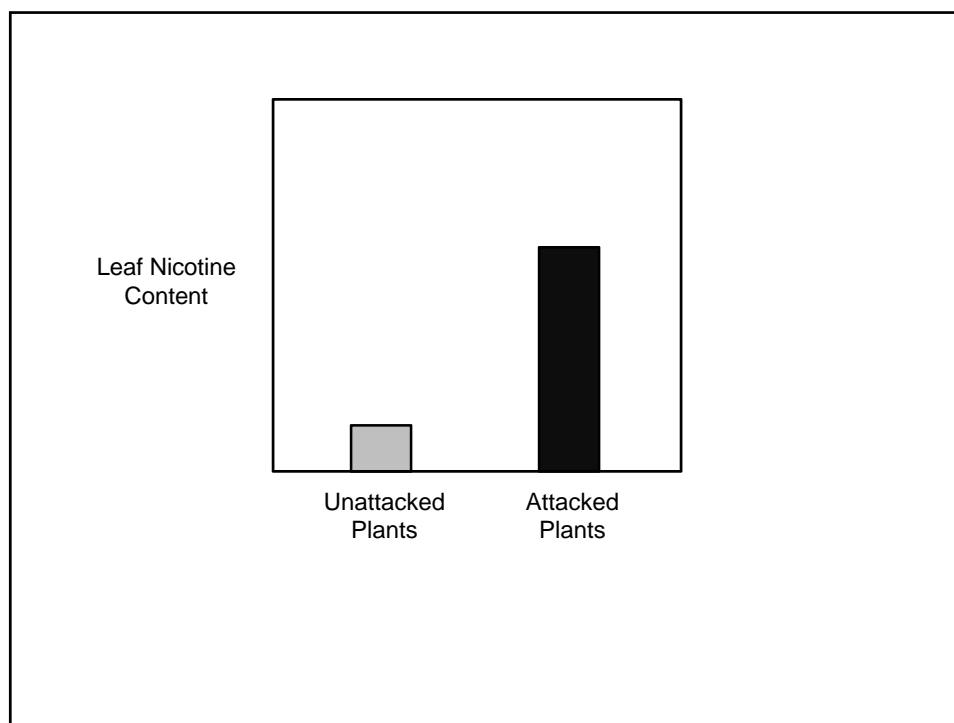
Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Carl Zeiss Promenade 10, D-07745 Jena, Germany

*You can't always get what you want, but if you try some time, you just might find, you get what you need...*

Mick Jagger

Unfortunately, a comprehensive understanding of internal processes is not sufficient to test the cost-benefit paradigm, because Darwinian fitness can also be influenced by processes external to the plant (Fig.

Baldwin, IT. 2001. *Plant Physiology* 127: 1449-1458.



### Mechanism

1. Herbivory induces jasmonic acid (JA) production.

CCCC/C=C/[C@H]1CC(=O)C[C@@H]1C(=O)O

2. JA to roots, stimulates nicotine synthesis.
3. Nicotine to shoots

## I. Plant Secondary Metabolites

### C. Ecology

#### Plant Compounds are Diuretics to Desert Herbivores

by Denise Dearing, Antonio Mangione and William Karasov

Many plant compounds are recognized deterrents and toxins to a variety of herbivores. The effect of such compounds on water balance of herbivores is virtually unexplored, yet many plant compounds cause diuresis by elevating urine production and decreasing urine concentration. Caffeine from coffee and black tea is probably the most familiar diuretic agent from plants. However, caffeine is not exceptional.

Plant products that cause diuresis in humans.

Diuretic Plant Extracts



## I. Plant Secondary Metabolites

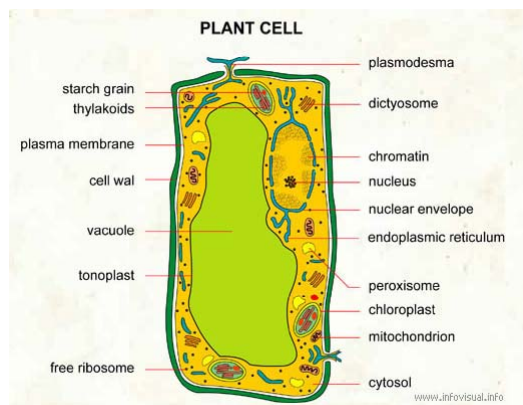
### C. Ecology



*Jasminum*

## I. Plant Secondary Metabolites

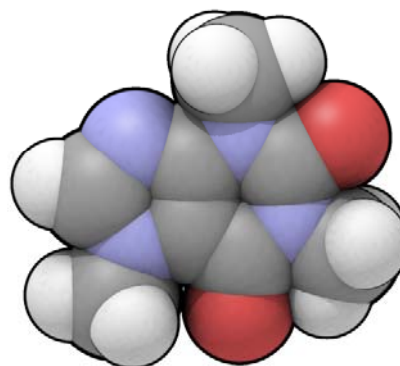
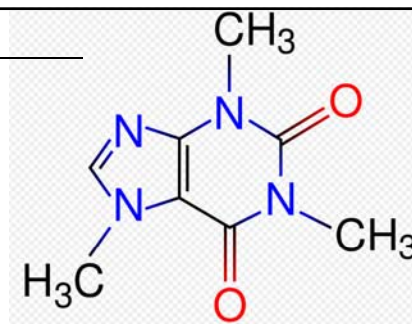
### D. Storage



## II. Caffeine case study

### Caffeine

- Coffea*, *Theobroma*, *Camellia*, *Cola*, etc.
- Psychoactive stimulant, diuretic
- Alkaloid





II. Caffeine case study

Caffeine

-syn. w/ guaranine

Species: *Paullinia cupana* (guarana' vine)  
 Family: Sapindaceae  
 Nativity: S. America



II. Caffeine case study

Caffeine

-syn. w/ theine

Species: *Camellia sinensis* (tea bush)  
 Family: Theaceae  
 Nativity: S. Asia





## II. Caffeine case study

### Caffeine

-syn. w/ mateine

Species: *Ilex paraguariensis*  
(yerba mate)

Family: Aquifoliaceae

Nativity: S. America.



## II. Caffeine case study

### Caffeine

-known as caffeine (orig. *kaffein*, from German *kaffee*)

Species: *Coffea arabica*  
(arabica coffee)

Family: Rubiaceae.

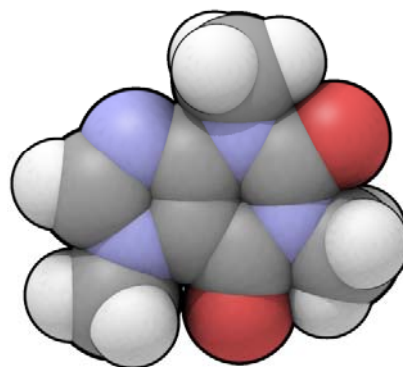
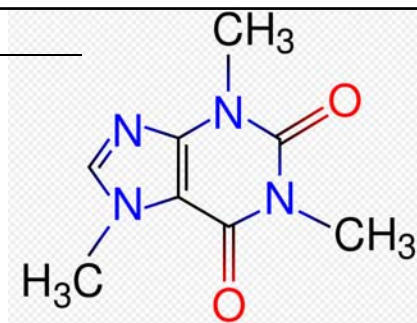
Nativity: NE Africa.



## II. Caffeine case study

### Alkaloids in general

- Secondary metabolites
- Nitrogenous
- Psychoactive (act on CNS)

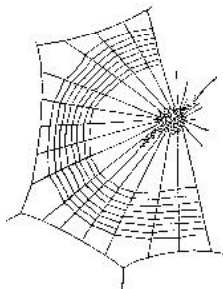


## II. Caffeine case study

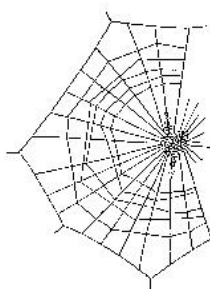
### Ecological role of alkaloids

Spider web manufacture when influenced by caffeine.

control



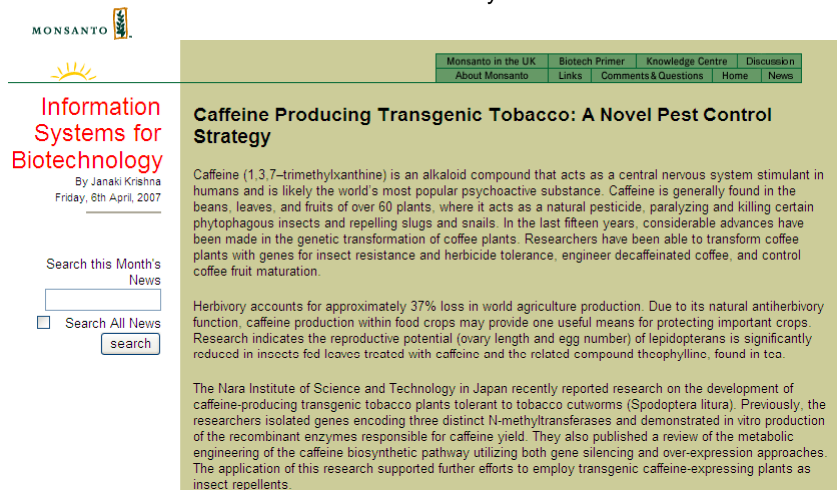
with caffeine



## II. Caffeine case study

### Ecological role of alkaloids

Caffeine's natural role noticed by Monsanto.



The screenshot shows a Monsanto website page. At the top left is the Monsanto logo. Below it is a search bar and a link to 'Information Systems for Biotechnology' by Janaki Krishna, dated Friday, 6th April, 2007. The main article title is 'Caffeine Producing Transgenic Tobacco: A Novel Pest Control Strategy'. The article text discusses caffeine as a natural pesticide and the development of transgenic tobacco plants. A navigation menu at the top right includes links for 'Monsanto in the UK', 'Biotech Primer', 'Knowledge Centre', and 'Discussion'.

**Monsanto**

Information Systems for Biotechnology  
By Janaki Krishna  
Friday, 6th April, 2007

Search this Month's News  
  
 Search All News

Monsanto in the UK | Biotech Primer | Knowledge Centre | Discussion  
About Monsanto | Links | Comments & Questions | Home | News

### Caffeine Producing Transgenic Tobacco: A Novel Pest Control Strategy

Caffeine (1,3,7-trimethylxanthine) is an alkaloid compound that acts as a central nervous system stimulant in humans and is likely the world's most popular psychoactive substance. Caffeine is generally found in the beans, leaves, and fruits of over 60 plants, where it acts as a natural pesticide, paralyzing and killing certain phytophagous insects and repelling slugs and snails. In the last fifteen years, considerable advances have been made in the genetic transformation of coffee plants. Researchers have been able to transform coffee plants with genes for insect resistance and herbicide tolerance, engineer decaffeinated coffee, and control coffee fruit maturation.

Herbivory accounts for approximately 37% loss in world agriculture production. Due to its natural antiherbivory function, caffeine production within food crops may provide one useful means for protecting important crops. Research indicates the reproductive potential (ovary length and egg number) of lepidopterans is significantly reduced in insects fed leaves treated with caffeine and the related compound theophylline, found in tea.

The Nara Institute of Science and Technology in Japan recently reported research on the development of caffeine-producing transgenic tobacco plants tolerant to tobacco cutworms (*Spodoptera litura*). Previously, the researchers isolated genes encoding three distinct N-methyltransferases and demonstrated in vitro production of the recombinant enzymes responsible for caffeine yield. They also published a review of the metabolic engineering of the caffeine biosynthetic pathway utilizing both gene silencing and over-expression approaches. The application of this research supported further efforts to employ transgenic caffeine-expressing plants as insect repellents.

[www.monsanto.co.uk](http://www.monsanto.co.uk)

## II. Caffeine case study

### Ecological role of alkaloids

Nitrogen availability is a major limiting factor in plant growth.



The screenshot shows an email header from the Science for Environment Policy DG Environment News Alert Service. It includes a globe icon, the text 'Science for Environment Policy', 'DG Environment News Alert Service', and the European Commission logo. The date '24 May, 2006' is also visible.

Science for Environment Policy  
DG Environment News Alert Service  
European Commission  
24 May, 2006

### Nitrogen Limitation Restricts CO<sub>2</sub> Absorption by Trees

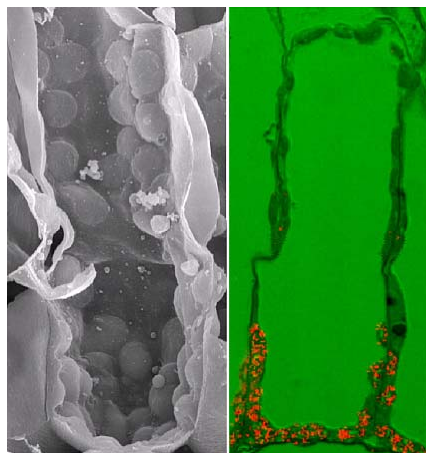
New research suggests that trees may not be able to limit climate change by storing rising atmospheric CO<sub>2</sub> as was previously believed. Limited availability of nitrogen in the soil, which will become more common as atmospheric CO<sub>2</sub> levels rise, may inhibit plant growth, which in turn would affect plants' accumulation of atmospheric CO<sub>2</sub>. Atmospheric CO<sub>2</sub> levels may therefore rise even faster than anticipated.

## II. Caffeine case study

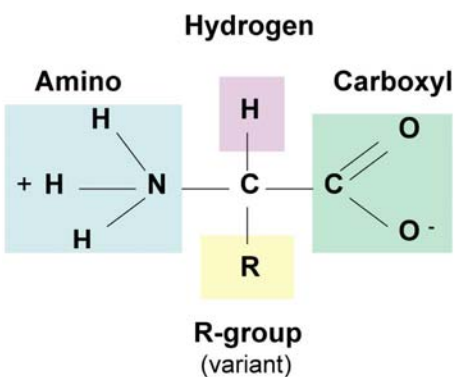
### Ecological role of alkaloids

Proteins (which drive biological reactions) require large amounts of N.

\*Proteins drive life processes (e.g., RuBisCO) and are important structural elements.



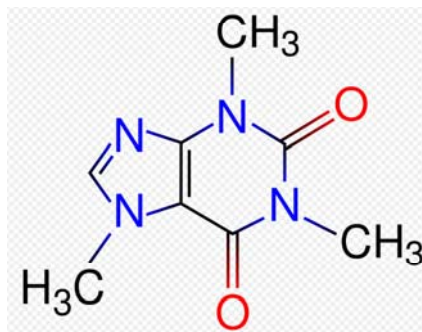
\*Amino acid structure



## II. Caffeine case study

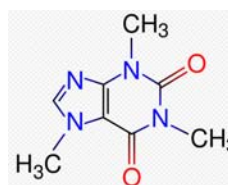
### Ecological role of alkaloids

\*Classic alkaloid composition exemplified by caffeine



Caffeine 's effects on CNS

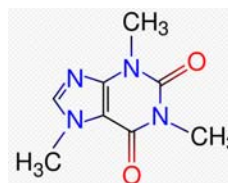
- Caffeine from coffee in blood w/in 5 min
- Stimulates heart
- Increases stomach acidity
- Increases urine output
- 10% rise in metabolic rate
- Mimics feelings assoc. w/ adrenaline



caffeine

Caffeine 's effects on CNS

- Caffeine from coffee in blood w/in 5 min
- Stimulates heart
- Increases stomach acidity
- Increases urine output
- 10% rise in metabolic rate



caffeine

- Mimics feelings assoc. w/ adrenaline

- Excess (1 g; 10 cups) can cause anxiety, headache, dizziness, insomnia, heart palpitations, delirium, 4% lower birth weights.

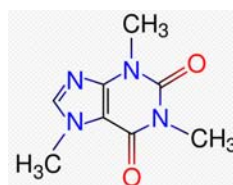
Caffeine 's effects on CNS

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- Increases urine output
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- Excess (1 g; 10 cups) can cause anxiety, headache, dizziness, insomnia, heart palpitations, delirium, 4% lower birth weights.

- Ranks as most widely used psychoactive drug worldwide (coffee, tea, additives to soft drinks)



caffeine

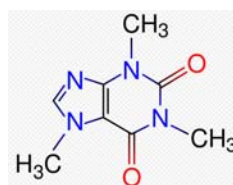
Caffeine 's effects on CNS

How?

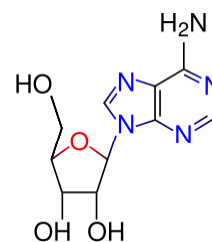
Antagonist of adenosine.

Adenosine:

- Attaches to brain cell receptors.
- Neurotransmitter inhibitor.
- Promotes sleep (accumulates in brain each waking hour).
- Suppresses arousal.



caffeine



adenosine

### Caffeine and Parkinson's prevention?

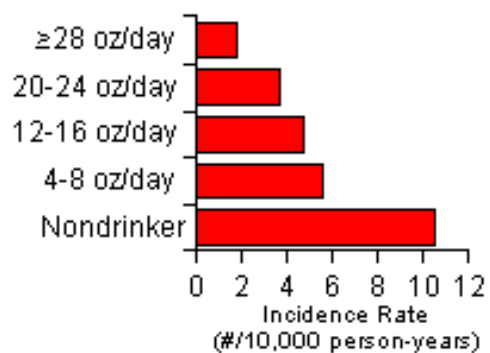
What is Parkinson's Disease?

*Journal of the American Medical Association*, March 24, 2000

- afflicts ca. 1-1.5 million people in the U.S., mostly 60 years +
- no known cause and no cure, just treatments
- symptoms of trembling arms and legs, trouble speaking, and difficulty coordinating movement
  
- Loss of dopamine-secreting neurons in the midbrain substantia nigra.
- Dopamine levels fall, and the balance between dopamine and other neurotransmitters disrupted, affecting muscular control

### Caffeine and Parkinson's prevention?

Honolulu Heart Program study of 8,000+ men over 30?



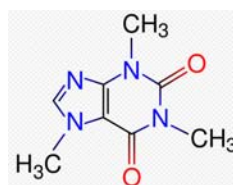
Mechanism: When adenosine receptors are blocked, dopamine levels increase.



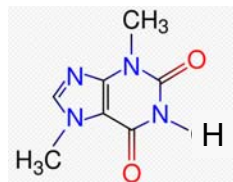
Caffeine and Theobromine are similar in structure and action

Table 1. Stimulant alkaloids in world's major stimulating beverages (Simpson 1986). Given in % weight. Amt. in particular beverage depends on how it is made.

Plant, part	Caffeine	Theobromine
Coffee, unroasted, dried seeds	1-1.5	--
tea, dried lvs.	2.5-4.5	--
Cacao, dried or fresh seeds	0.6-0.8	1.7-2.4
Kola, fresh seeds	2.0	--
Guarana, dried fruit	3.0-4.5	--



caffeine

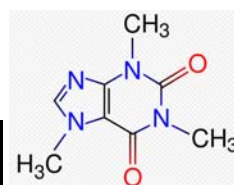


theobromine

Caffeine in some beverages

Table 2. Caffeine quantities in select beverages.

Drink	Caffeine (mg)
<b>Coffee (Starbucks)</b>	
12 oz drip	240
1 oz espresso	?
12 oz drip decaf	19

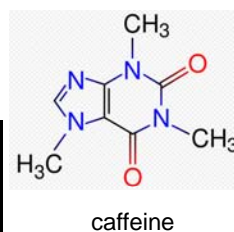


caffeine

Caffeine in some beverages

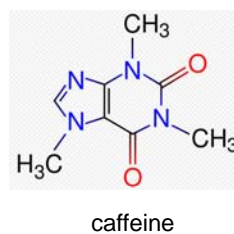
Table 2. Caffeine quantities in select beverages.

Drink	Caffeine (mg)
<b>Coffee (Starbucks)</b>	
12 oz drip	240
1 oz espresso	75
12 oz drip decaf	19



**Table 6.** Average caffeine content in products (most amounts from the Center for Science in the Public Interest, 2007; chocolate amounts from Simpson and Orzogoly 1995).

Product	Caffeine (to the nearest mg)
<b>Coffee (Starbucks)</b>	
12 oz drip coffee	240
1 oz espresso	75
12 oz drip decaf coffee	19
<b>Tea (various)</b>	
12 oz brewed tea	80 (60-180)
12 oz Nestea	26
12 oz Snapple	14-32
<b>Cocoa and chocolate (various)</b>	
12 oz, from powder	14 (4.5-20)
1 oz baking choc	35
1 oz dark choc	20
1 oz milk choc	6
<b>Soda (various)</b>	
8.3 oz Red Bull	80
12 oz Jolt Cola	72
12 oz Mountain Dew	54
12 oz Dr. Pepper	42
12 oz Pepsi	38
12 oz Coca-Cola Classic	35



III. *Coffea*

Coffee intro

Coffee is world's second most traded commodity.

Q: What are the top 5 traded world commodities?  
 by [Commodity.com](#)  
 A:  
 1 Oil  
 2 Wheat  
 3 Iron ore  
 4 Soybeans  
 5 Coffee



*Coffea arabica*  
(arabica coffee)

Table 3. Production of top 3 stimulant beverages.

Top 3 continents	Total (MT)
<b>Coffee</b>	5,919
1. S Amer	
2. Africa	
3. N & C Amer	
<b>Tea</b>	2,473
1. Asia	
2. Africa	
3. S Amer	
<b>Cocoa</b>	2,329
1. Africa	
2. S Amer	
3. Asia	

III. *Coffea*

Coffee breeding

Coffee Biotech Group (Campinas, Brazil)



III. Coffea

Coffea berries and flowers



III. Coffea

Coffea berries and flowers



III. Coffea

Coffea seeds



III. Coffea

Coffea seeds





### III. *Coffea*

#### Coffee origins

*Coffea arabica*, old world tropical/subtropical crop grown at mid to upper-middle elevations

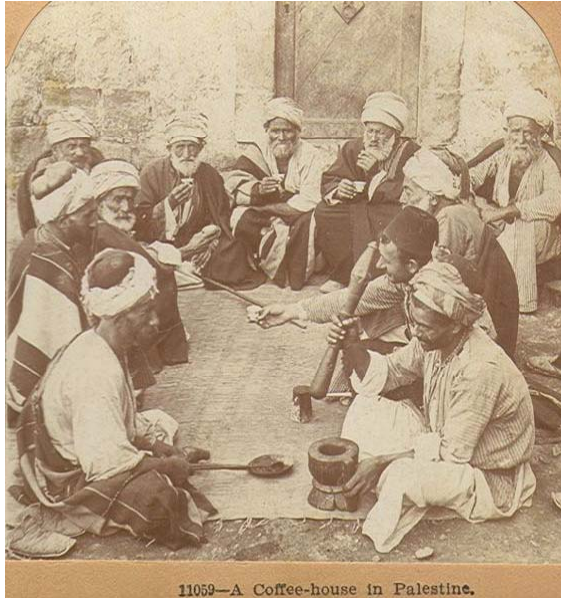
- Relatively few Africans drink it.
- Traditionally chewed leaves & fruits.
- Relief of fatigue, hunger on hunts.



Farmer in Ethiopia

#### Coffee (the drink) developed in Yemen

Arrival in Yemen 13-14 century, where it was first brewed (hence, *Coffea arabica*).



ca. 1900

11059—A Coffee-house in Palestine.

### Coffee timeline

1. First brewed in Yemen 13-14 century (hence, *Coffea arabica*).
2. Arabia to Egypt by 1510.
3. To Italy & Europe by 1616.
4. Vienna priests threatened by “coffee culture”, but Pope Clement VIII would not ban coffee.
5. To England by 1650 and coffee houses became important socio-politico institutions.
6. Europe looked to break Arabian monopoly on production.  
(*Arabians killed embryos in seeds before export*).

### Spread of Coffee production

- Dutch obtained live seeds from Mocha (Red Sea Coast, Yemen, 1706)
- Throughout Dutch colonies in Indonesia (e.g., Java) and to S America by 1717.
- Today, Brazil is world's leading producer.

