Phytomelatonin: A Defense against Abiotic and Biotic Stress

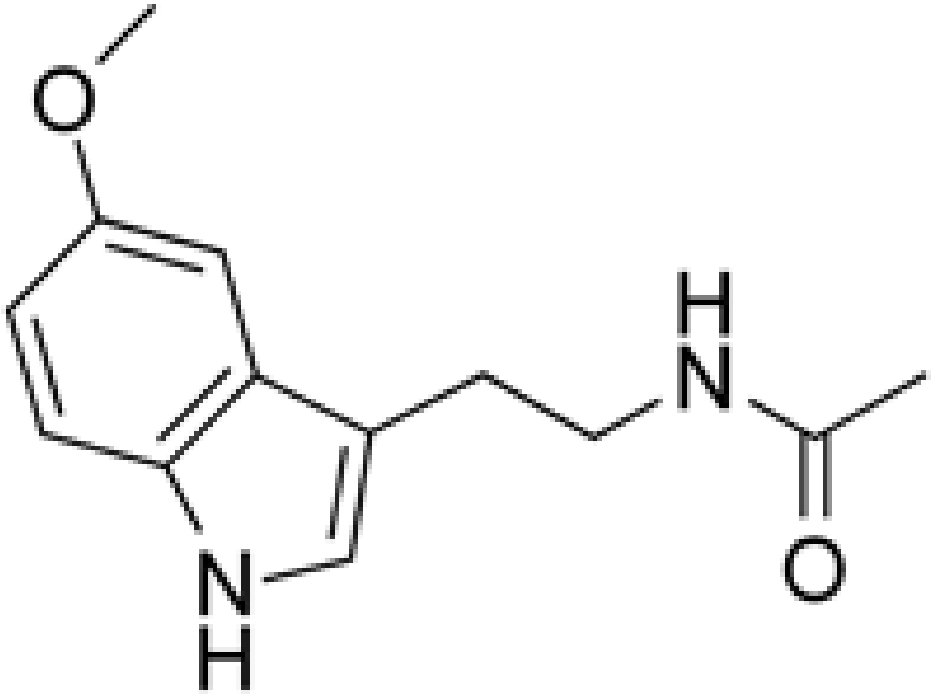
Russel J. Reiter, Ph.D., M.D.(*hc*), D.Sc.(*hc*)
Professor of Cell Biology
Department of Cellular and Structural Biology
University of Texas Health Science Center
San Antonio, Texas

reiter@uthscsa.edu

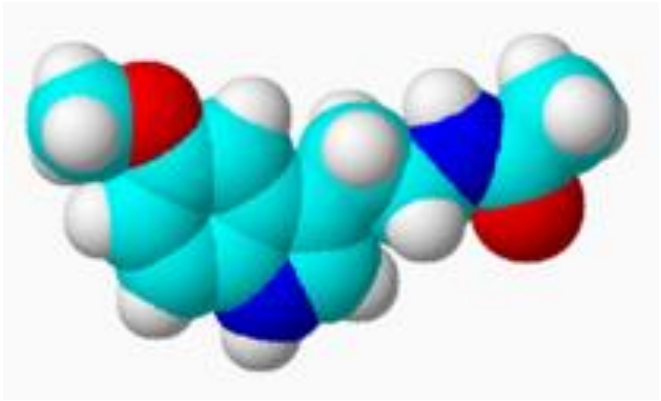




Melatonin (N-acetyl-5-methoxytryptamine)



"mela" from melanin
"tonin" from serotonin



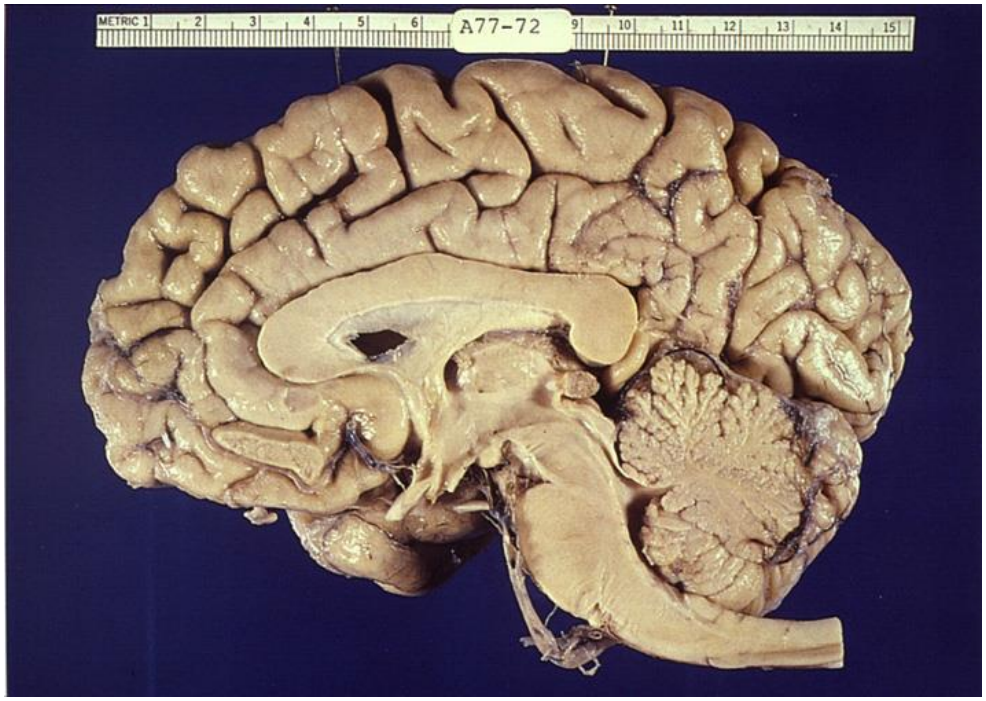
Melatonin:

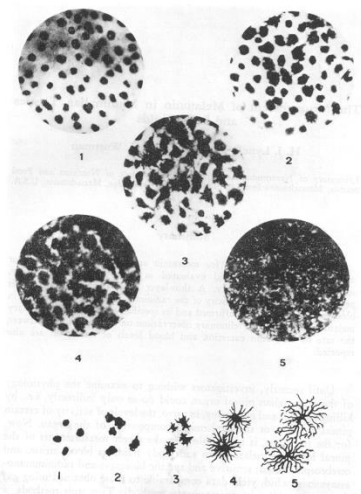
***i*) an extremely old molecule**

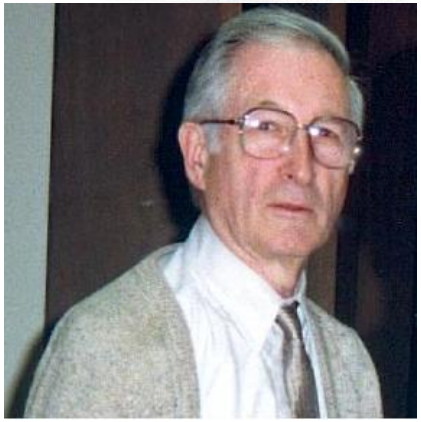
***ii*) exists in every species**

***iii*) structure never changed thru out evolution**

Lerner AB, Case JD, Takahashi Y, Lee Y, Mori W. Isolation of the pineal factor that lightens melanocytes. J Am Chem Soc 1958; 8: 2587







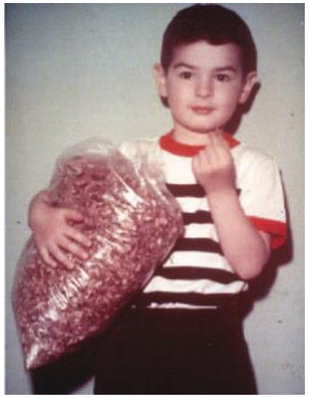
Aaron B. Lerner, M.D.
Dermatologist



Not fed pineal tissue



Fed pineal tissue

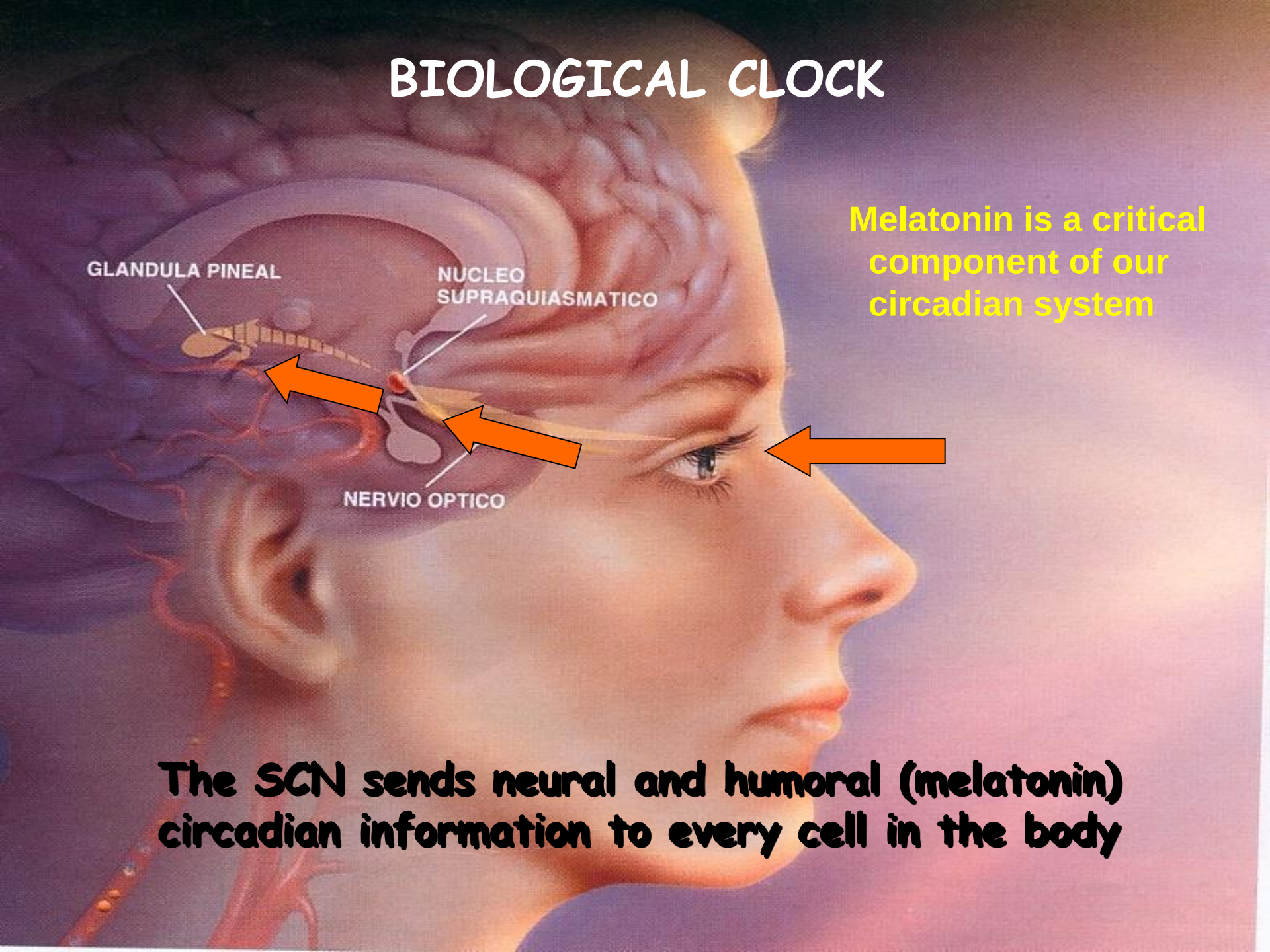


When melatonin was discovered it was thought to be

i) exclusively of vertebrate origin

ii) exclusively located in the pineal gland

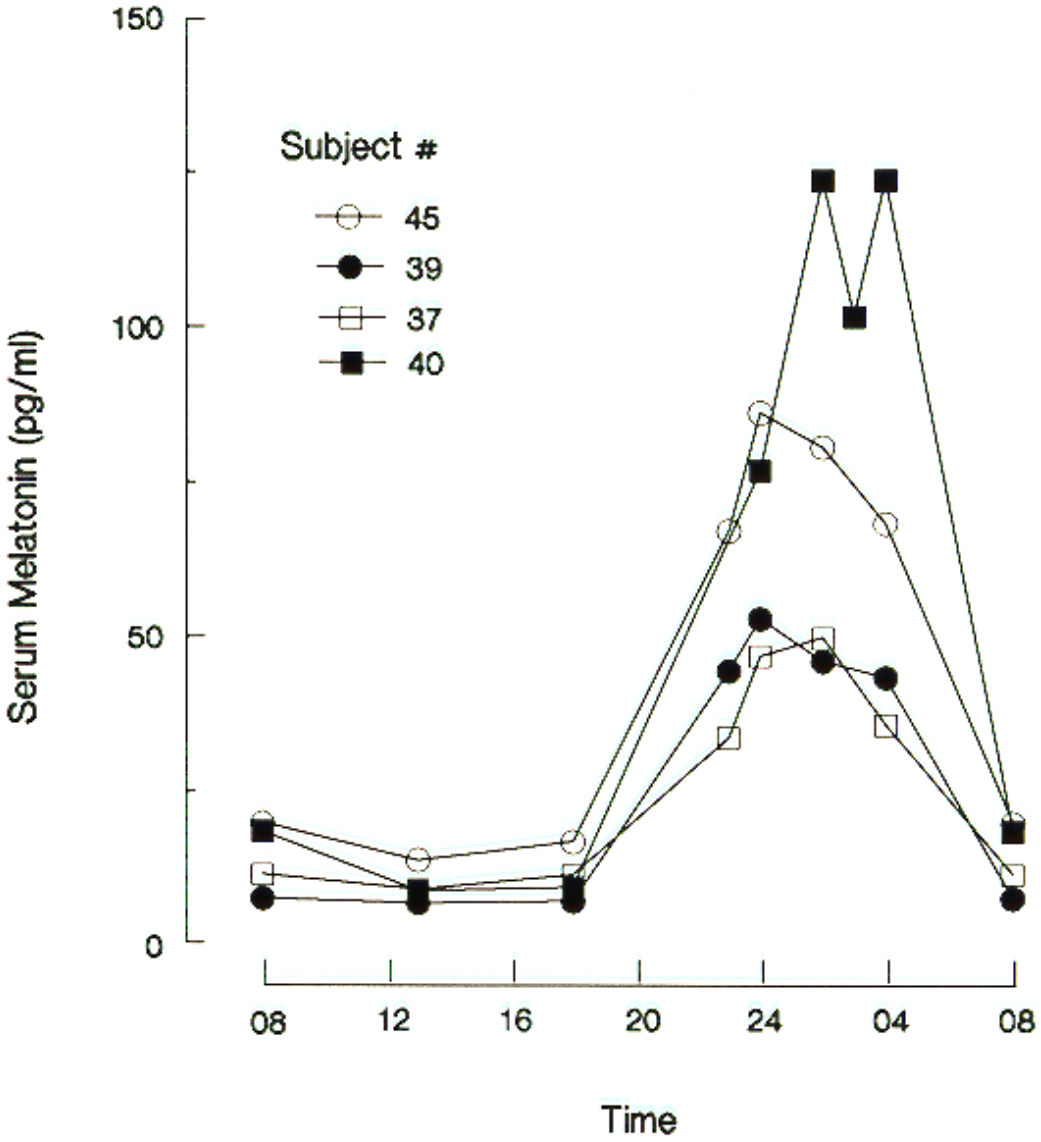
BIOLOGICAL CLOCK



Melatonin is a critical component of our circadian system

The SCN sends neural and humoral (melatonin) circadian information to every cell in the body

In all animal species (algae to humans), melatonin exhibits a circadian rhythm



Melatonin is known as chemical "expression of darkness"

Many other organs produce melatonin but do not release it into the blood

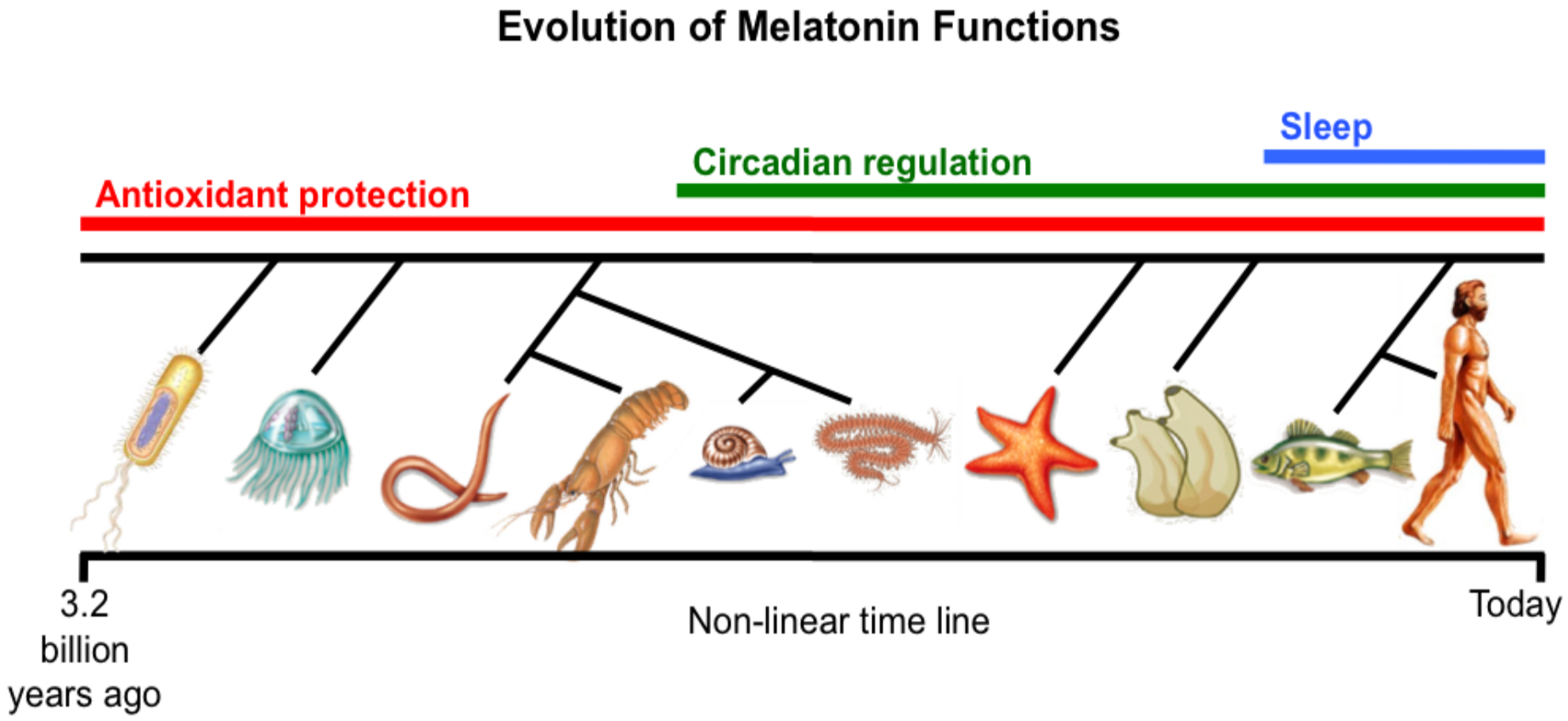
Discovery of Melatonin in Plants

Dubbels R, Reiter RJ et al. Melatonin in edible plants identified by radioimmunoassay and high performance liquid chromatography-mass spectrometry. J Pineal Res 1995; 18: 28-31

Hattori A, Reiter RJ et al. Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. Biochem Mol Biol Int 1995; 35: 627-634

Prokaryotes (bacteria) produce melatonin as an antioxidant

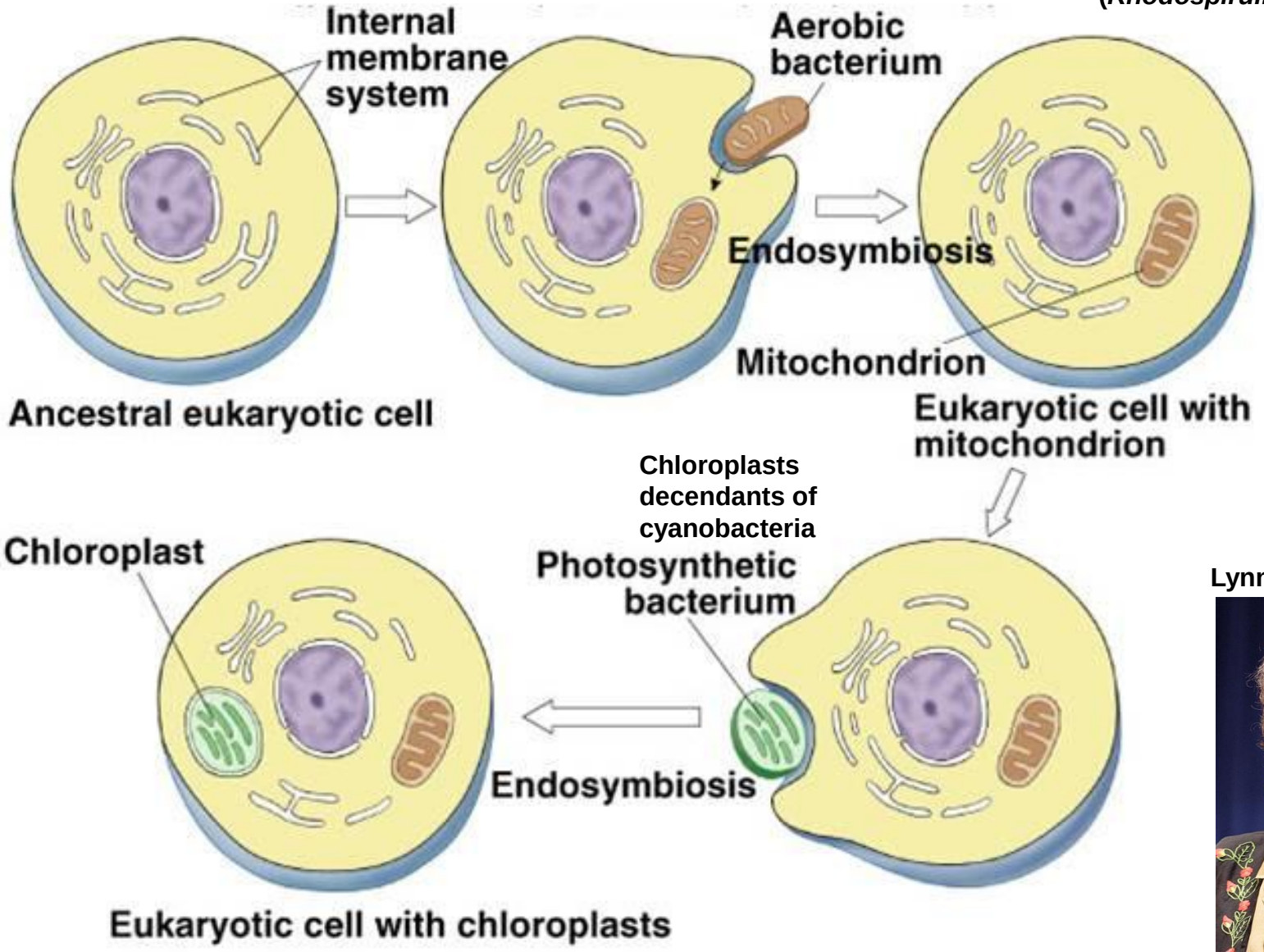
Melatonin regulation of sleep is a more recent development



The first function of melatonin was as an antioxidant

Endosymbiotic theory of the origin of mitochondria and chloroplasts

Mitochondria from purple non-sulfur bacterium (*Rhodospirullum rubrum*)



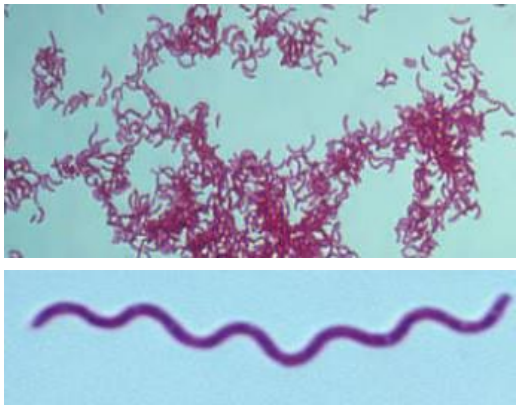
Lynn Margolis, PhD



Cyanobacteria

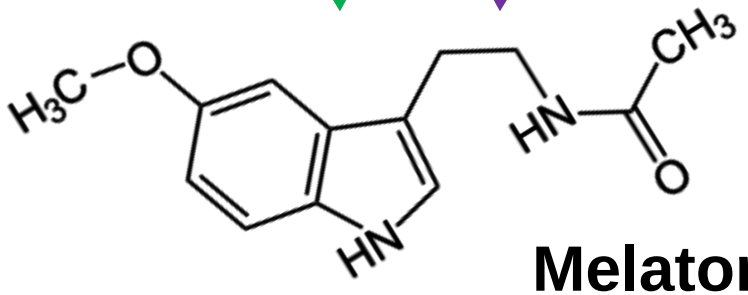


Purple non-sulfur bacteria



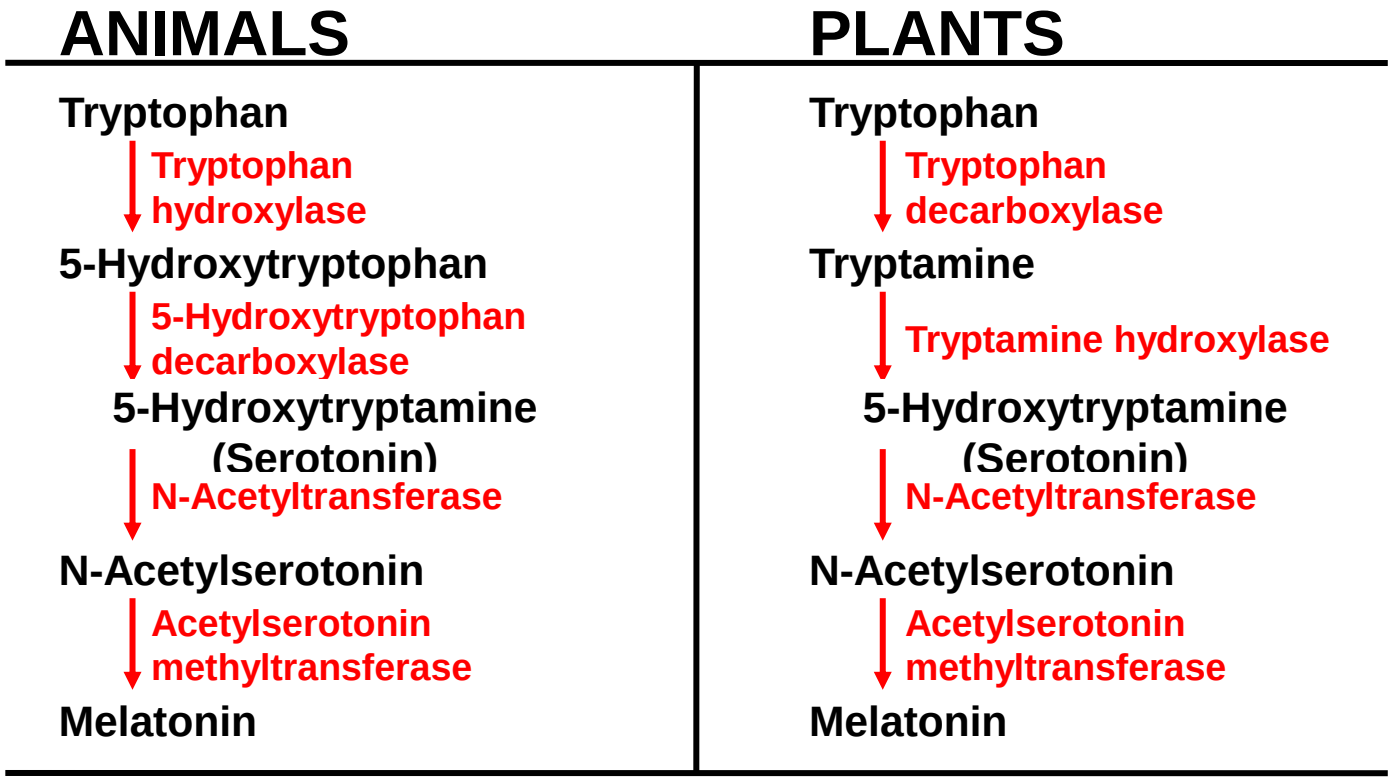
(engulfed and became chloroplasts)

(engulfed and became mitochondria)

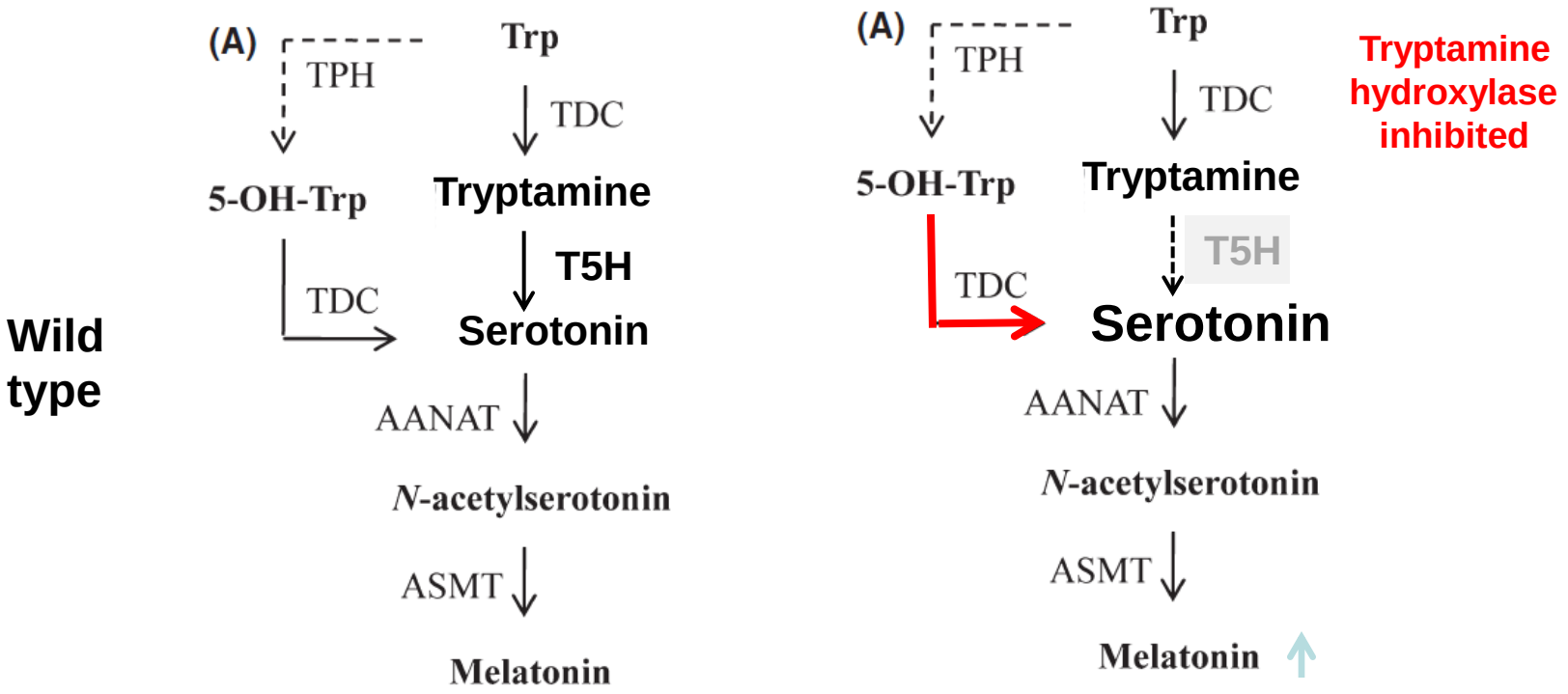


Melatonin

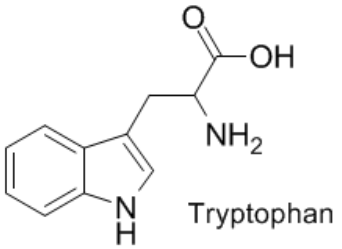
Comparison of melatonin synthesis in animals and plants



Unexpectedly, transcriptional inhibition of tryptamine hydroxylase does not suppress melatonin levels in plants

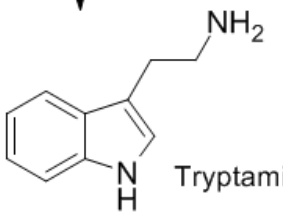


Plants have both the chloroplast (plant) pathway and the mitochondrial (plant and animal) pathway of melatonin synthesis



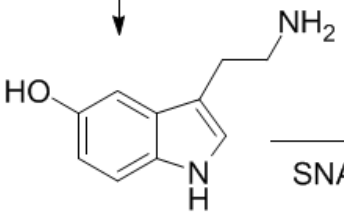
Tryptophan

TD



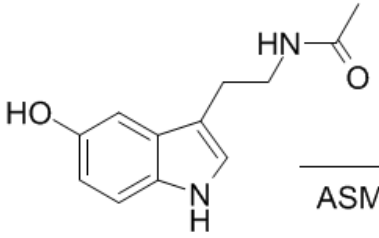
Tryptamine

T5H



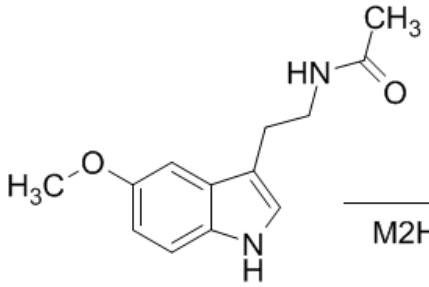
Serotonin

SNAT



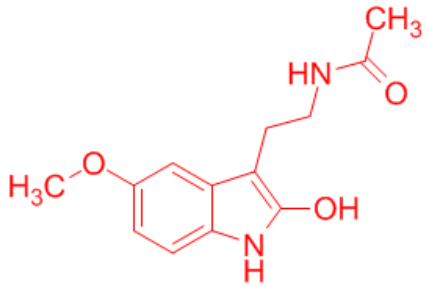
N-Acetylserotonin

ASMT

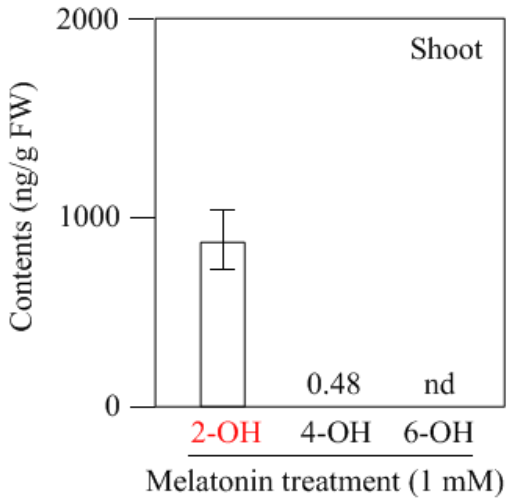


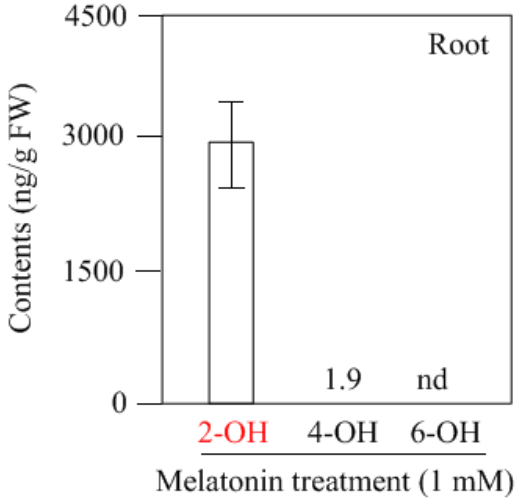
Melatonin

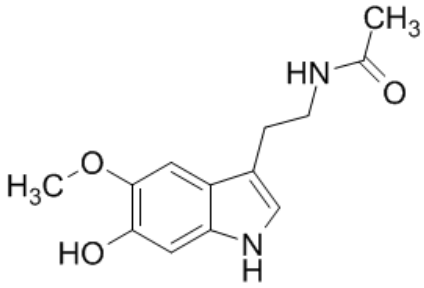
M2H



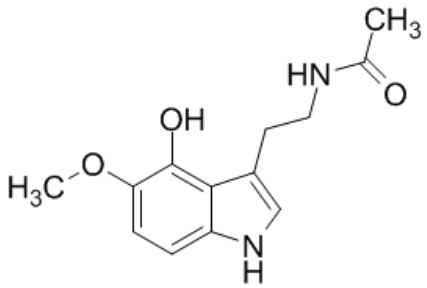
2-Hydroxymelatonin (2-OH)





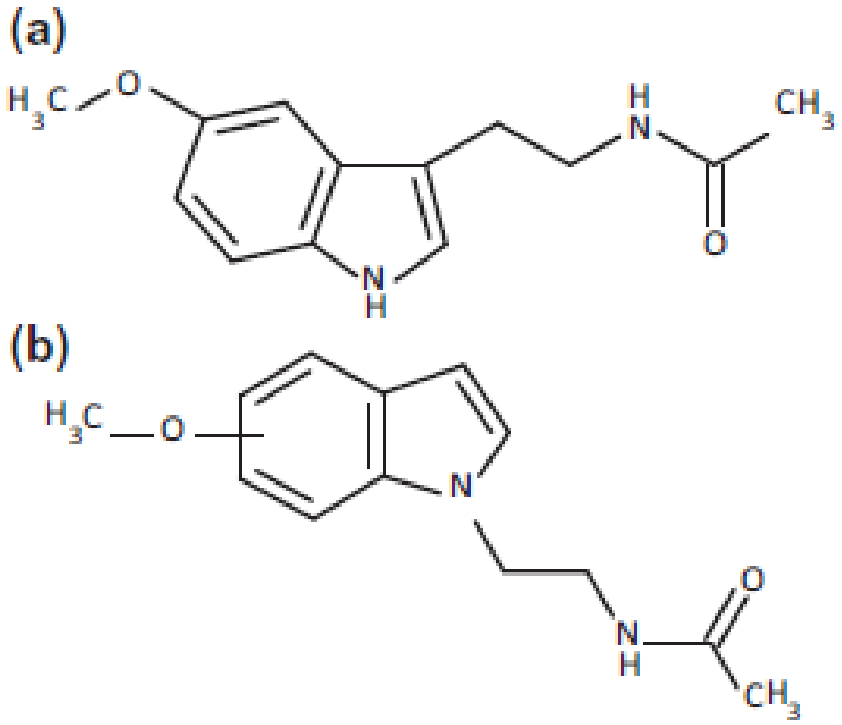


6-Hydroxymelatonin (6-OH)



4-Hydroxymelatonin (4-OH)

Melatonin isomers have been identified in plants



Melatonin

Melatonin isomers

(cannot be easily distinguished from melatonin)

The functional significance of isomers in plants remains uninvestigated

High lipid content of seeds make them vulnerable to oxidative damage

Life Sciences 67 (2000) 3023–3029

High levels of melatonin in the seeds of edible plants
Possible function in germ tissue protection

Lucien C. Manchester[b], Dun-Xian Tan[a], Russel J. Reiter[a,*], Won Park[b], Kanishka Monis[b], Wenbo Qi[a]

Melatonin concentrations in seeds of 15 edible plants

Common name	Scientific name	Melatonin content (ng/g dry seed)
Milk thistle	*Silybum marianum*	2
Poppy	*Popaver somniferum*	6
Anise	*Pimpinela anisum*	7
Coriander	*Coriandrum sativum*	7
Celery	*Apium graveolens*	7
Flax	*Linum usitatissimum*	12
Green cardamom	*Elettaria cardamomum*	15
Alfalfa	*Medicago sativum*	16
Fennel	*Foeniculum vulgare*	28
Sunflower	*Helianthus annuus*	29
Almond	*Prunus amygdalus*	39
Fenugreek	*Trigonella foenum-graecum*	43
Wolf berry	*Lycium barbarum*	103
Black mustard	*Brassica nigra*	129
White mustard	*Brassica hirta*	189

Melatonin concentrations in organs of tomato plants

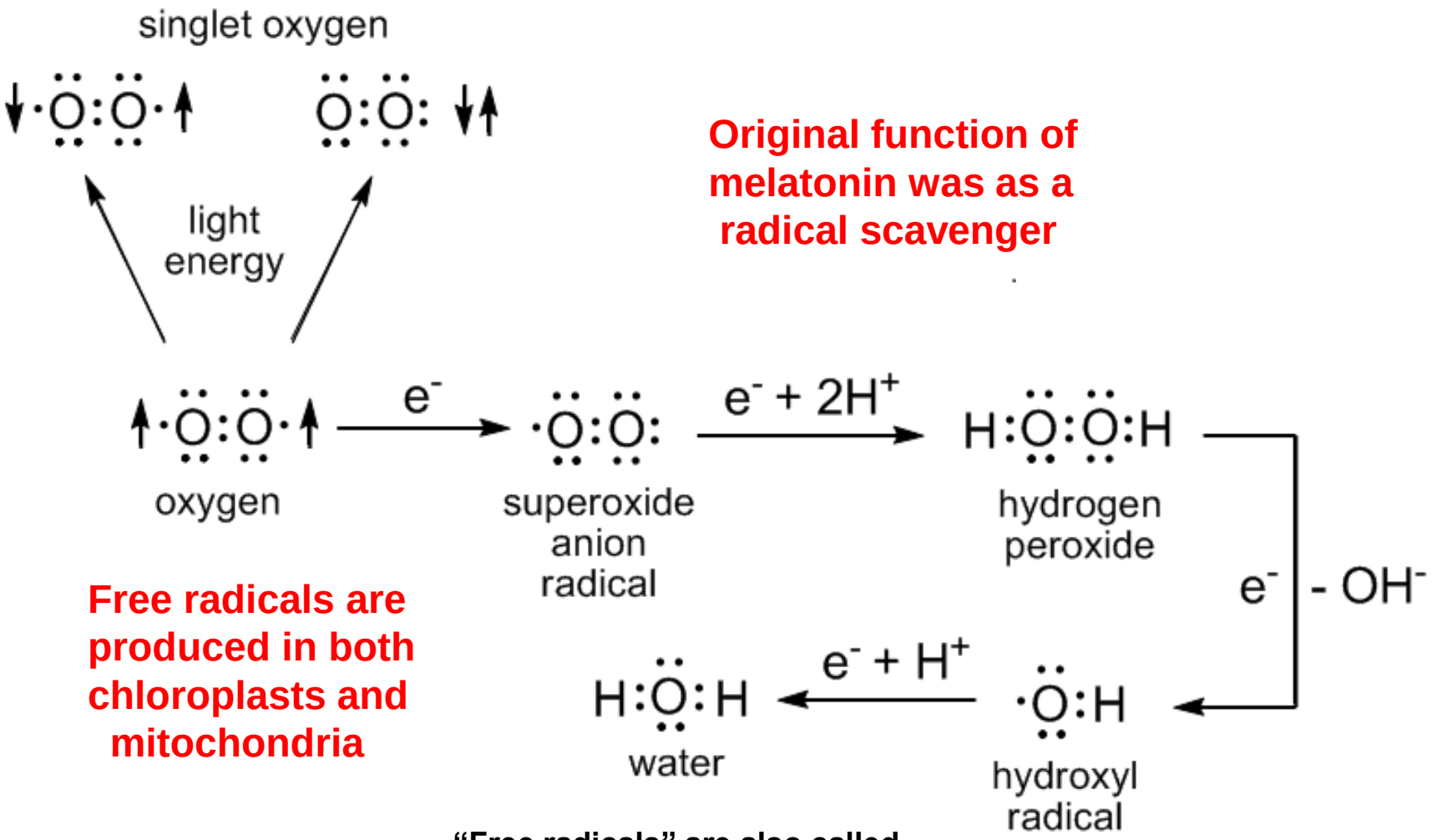
Organ	Melatonin (ng/g FW)
Leaflet	4.6
Leaf stems	2.0
Stems	5.3
Roots	3.1
Flowers	2.8
Red fruits	2.5
Seedlings	22.7
Seeds	39.4

Melatonin in corn. All 58 varieties were collected at the same site, i.e., the Institute of Rice, Fujian Academy of Agricultural Sciences, Fuzhou, China

Variety	Mel (μg/kg)	Variety	Mel (μg/kg)	Variety	Mel (μg/kg)
Corn 056	2034	Corn 031	41	Corn-N06	21
Corn 049	1981	Corn 129	41	Corn-N14	21
Corn 054	1110	Corn 143	39	Corn 159	20
Corn 115	983	Corn 057	37	Corn 042	19
Corn 046	803	Corn-N24	37	Corn-N13	19
Corn-N01	803	Corn-N04	35	Corn 150	18
Corn 047	614	Corn 155	35	Corn-N07	18
Corn-N02	614	Corn 038	33	Corn-N11	18
Corn 055	529	Corn 004	32	Corn-N18	18
Corn 163	446	Corn-N21	32	Corn 034	17
Corn 139	425	Corn 148	29	Corn-N19	17
Corn 032	325	Corn 007	28	Corn 140	16
Corn-N03	325	Corn-N23	27	Corn-N17	16
Corn 151	233	Corn-037	26	Corn-N22	16
Corn 033	172	Corn-N05	26	Corn-N16	15
Corn 142	142	Corn 040	25	Corn 009	14
Corn 149	81	Corn-N12	24	Corn-N10	14
Corn 144	78	Corn-N08	23	Corn-N09	11
Corn 035	62	Corn-N15	22		
Corn 004	60	Corn-N20	22		

Different concentrations may be real or a consequence of maturation stage, time of day harvested, environmental factors, duration of storage, etc.

The toxicity of oxygen



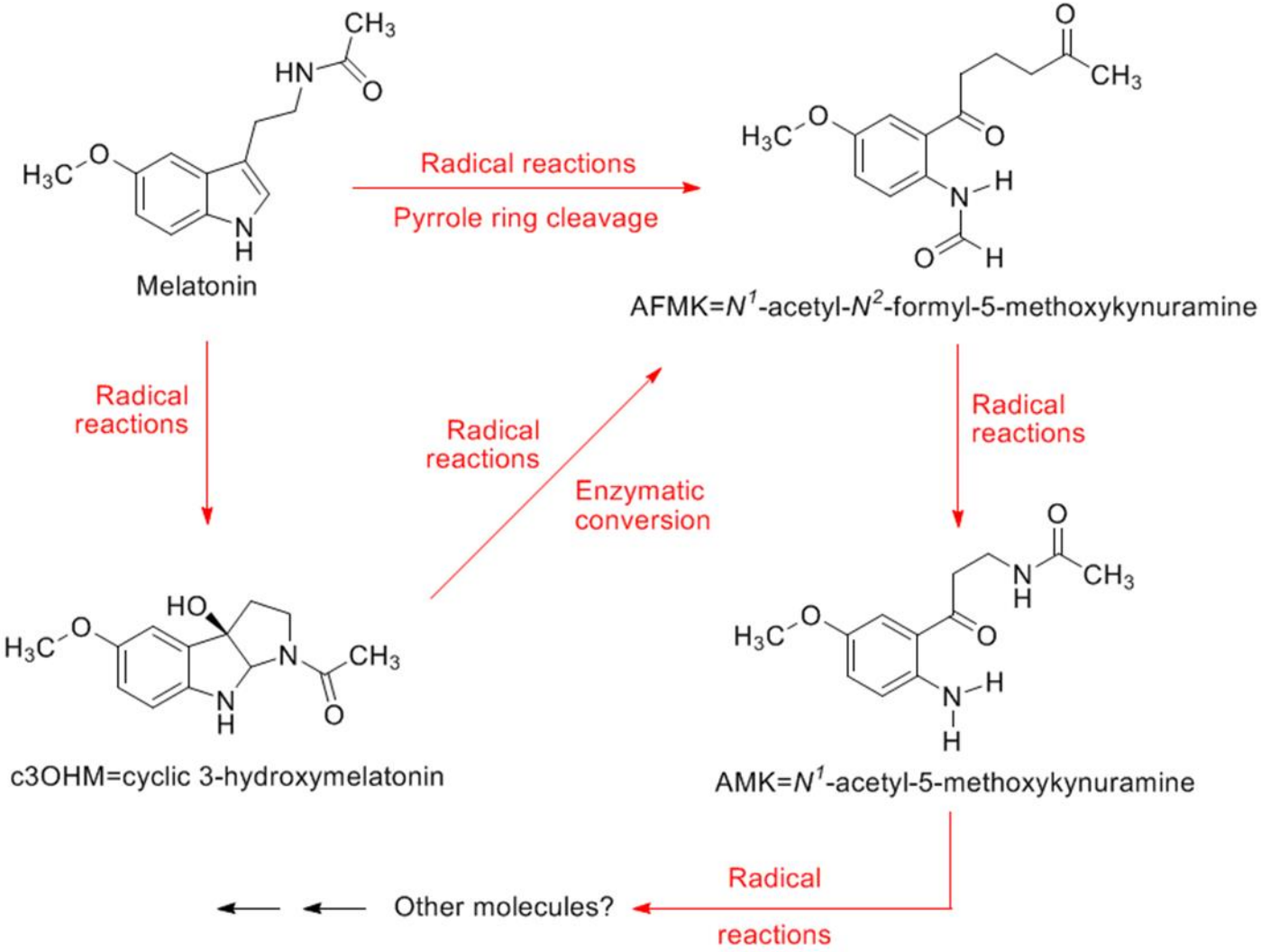
Original function of melatonin was as a radical scavenger

Free radicals are produced in both chloroplasts and mitochondria

"Free radicals" are also called reactive oxygen species (ROS)

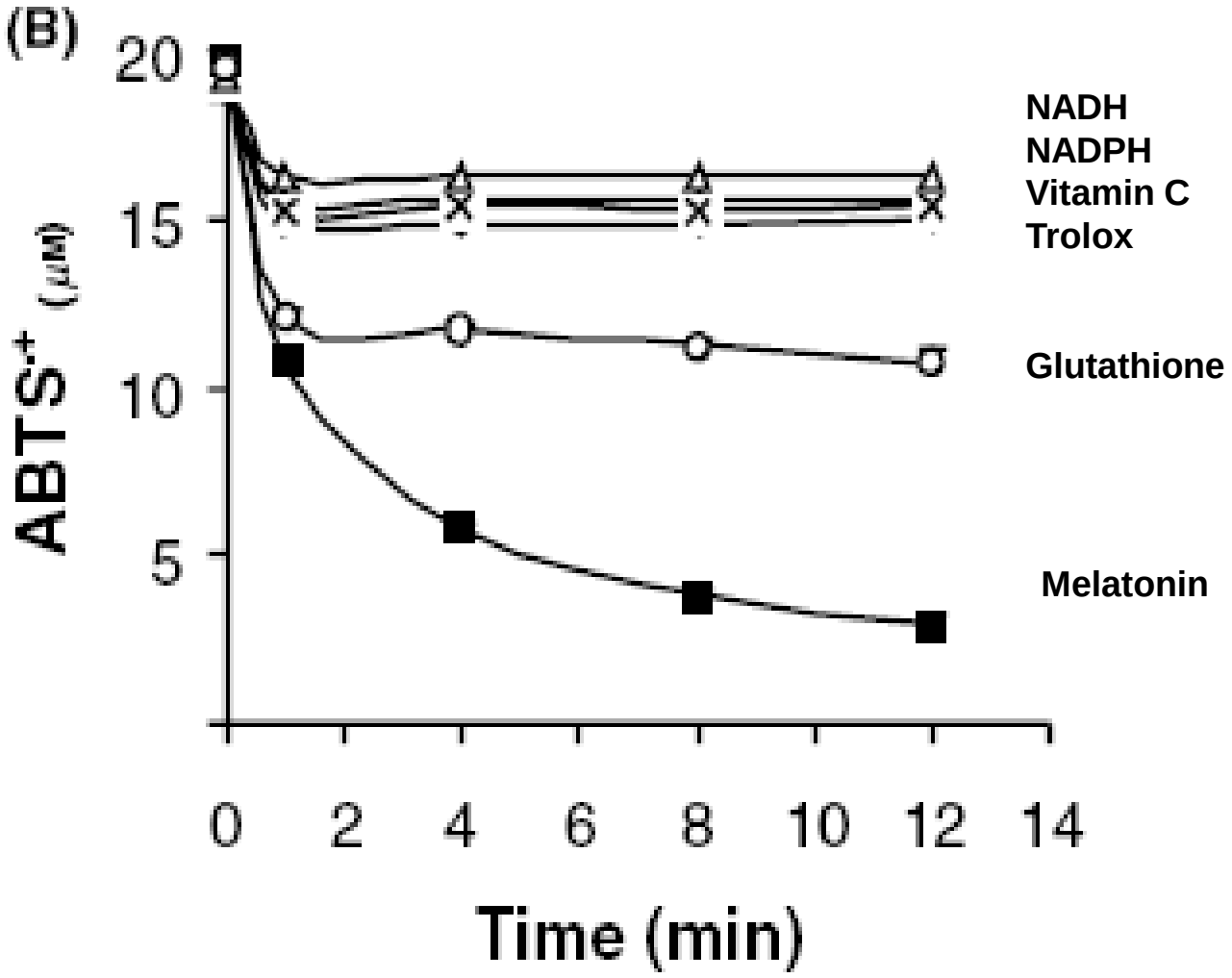
Reiter et al, 2015

Melatonin's antioxidant cascade



Primary, secondary and tertiary metabolites of melatonin are radical scavengers

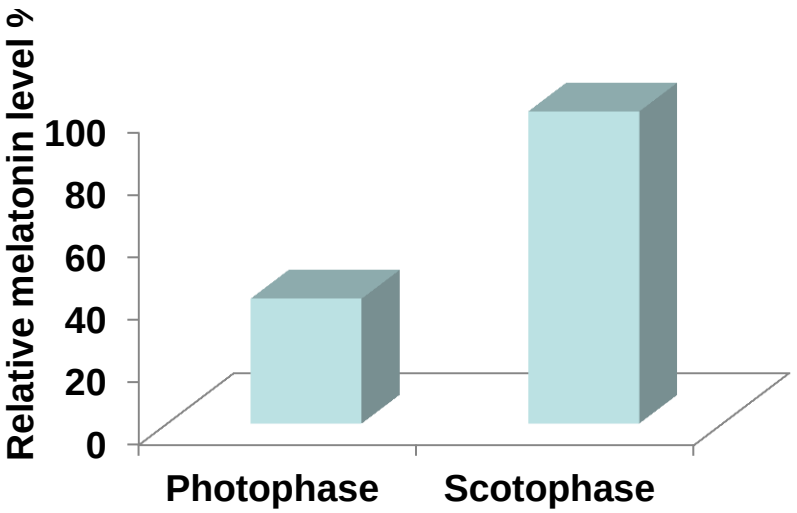
Time dependence of ABTS+ scavenging by melatonin and some classic antioxidants (antioxidant concentration = 10 μM)



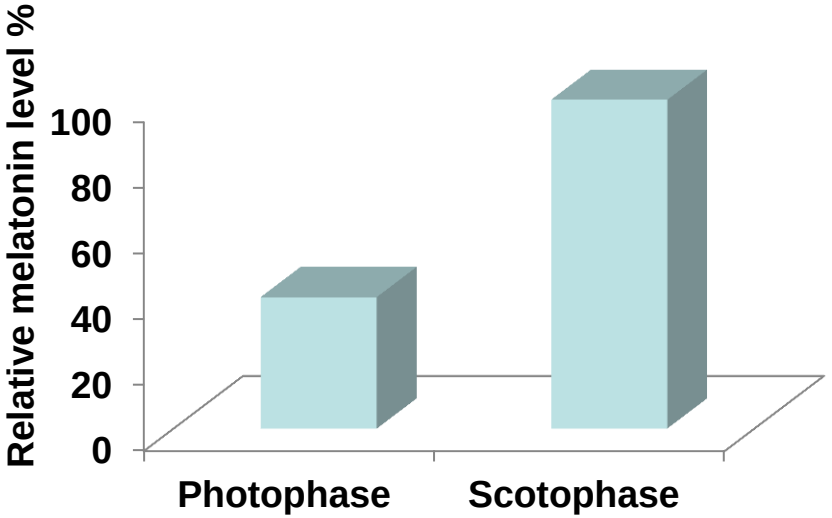
Antioxidants are designed to defeat free radicals

Tan et al, 2010

Vascular plants may not have a day:night melatonin rhythm

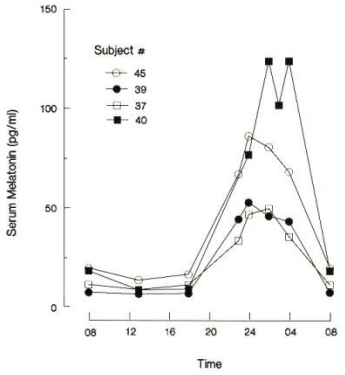


Rhodospirillum rubrum
(Photosynthetic bacterium)



Dinoflagellate Gonyaulax polyedra
(Unicellular organism, algae)

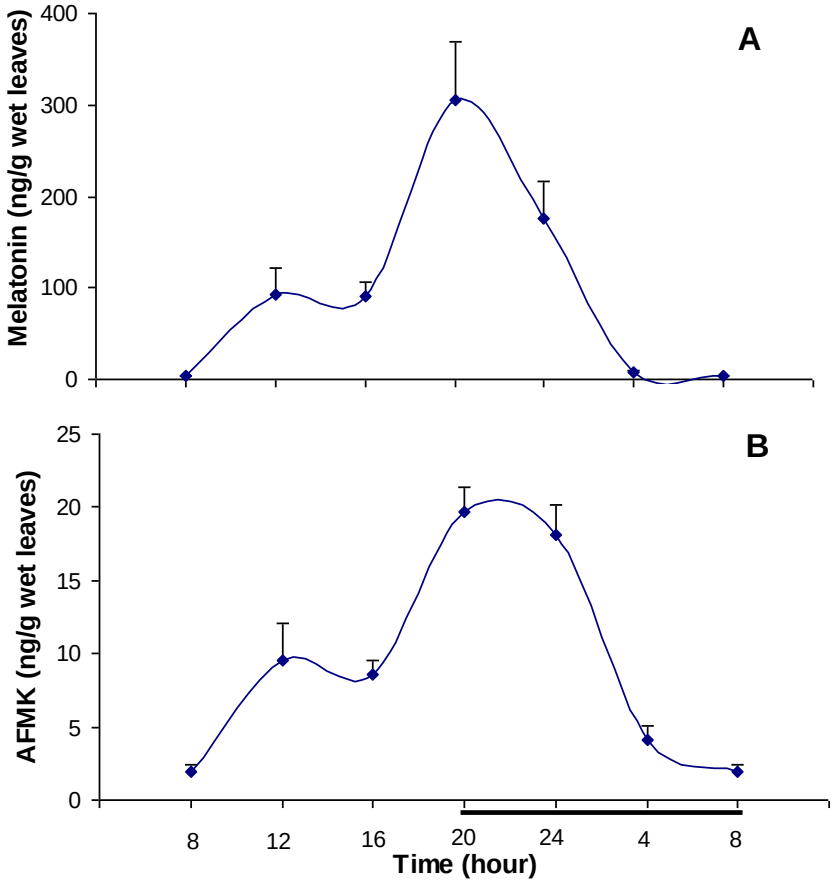
Day;night melatonin rhythm in vertebrates



In vascular plants melatonin fluctuations may be induced by metabolic activity or by environmental stressors



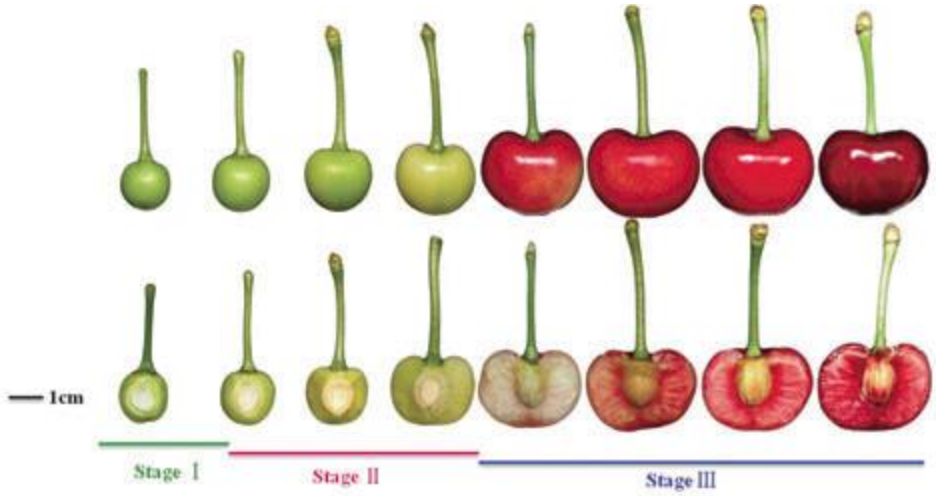
Melatonin and AFMK rhythms in the water hyacinth (*Eichhornia crassipes*)



Evidence that melatonin functions as an antioxidant in plants

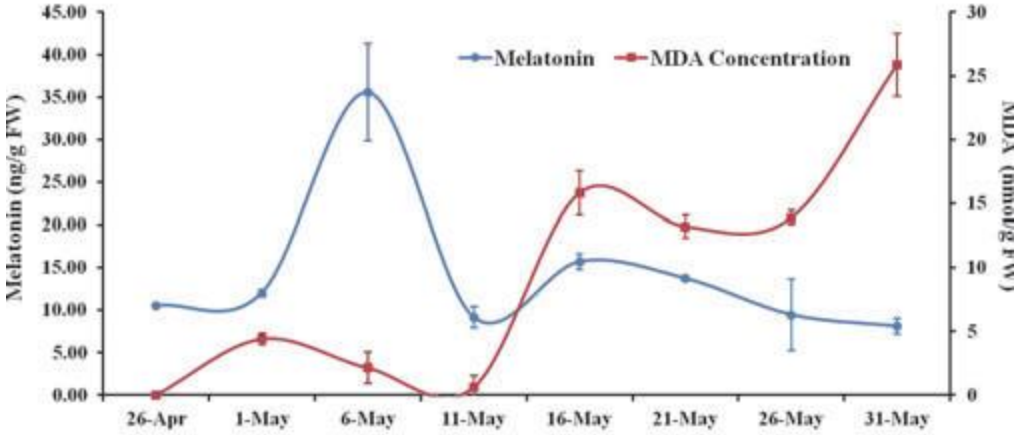
When melatonin scavenges radicals it is converted to AFMK

Tan, Reiter, 2007

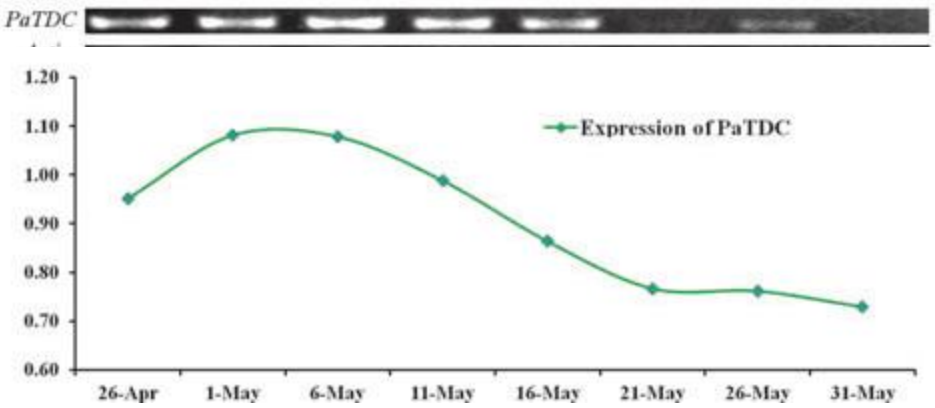


Melatonin functions as an antioxidant during fruit development

Hengdang sweet cherries



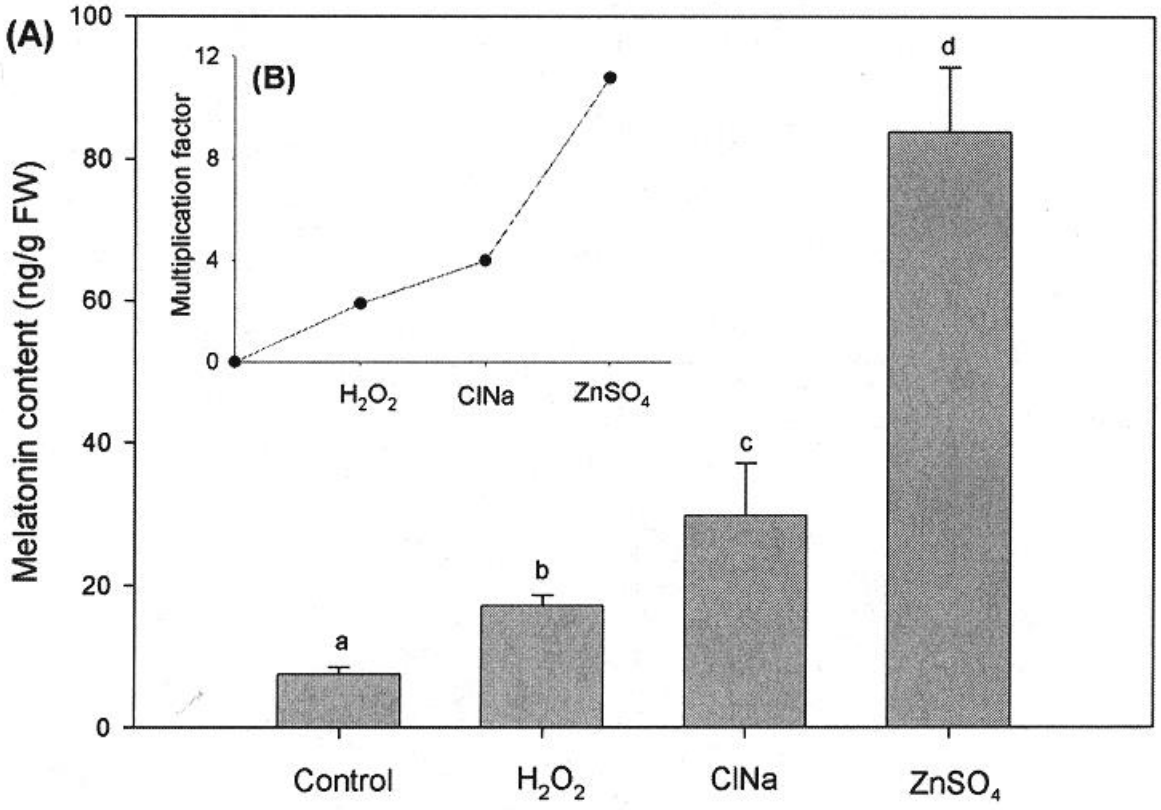
Melatonin and lipid peroxidation (MDA) levels during cherry maturation



Tryptophan decarboxylase gene expression (rate limiting enzyme)

Zhao, Reiter, Jin, 2012

Melatonin concentrations increase in lupin (bean) plants upon exposure (24 h) to chemical toxins



Compensatory increases in melatonin

Salty soil is becoming a major problem in some areas

Hydrogen peroxide = 10 mM
Sodium chloride = 10 mM
Zinc sulfate = 1 mM

Arnao, i2015

Melatonin concentrations in roots of lupin (bean) plants as a function of water stress or addition of melatonin to the soil

Drought stress for 7 days

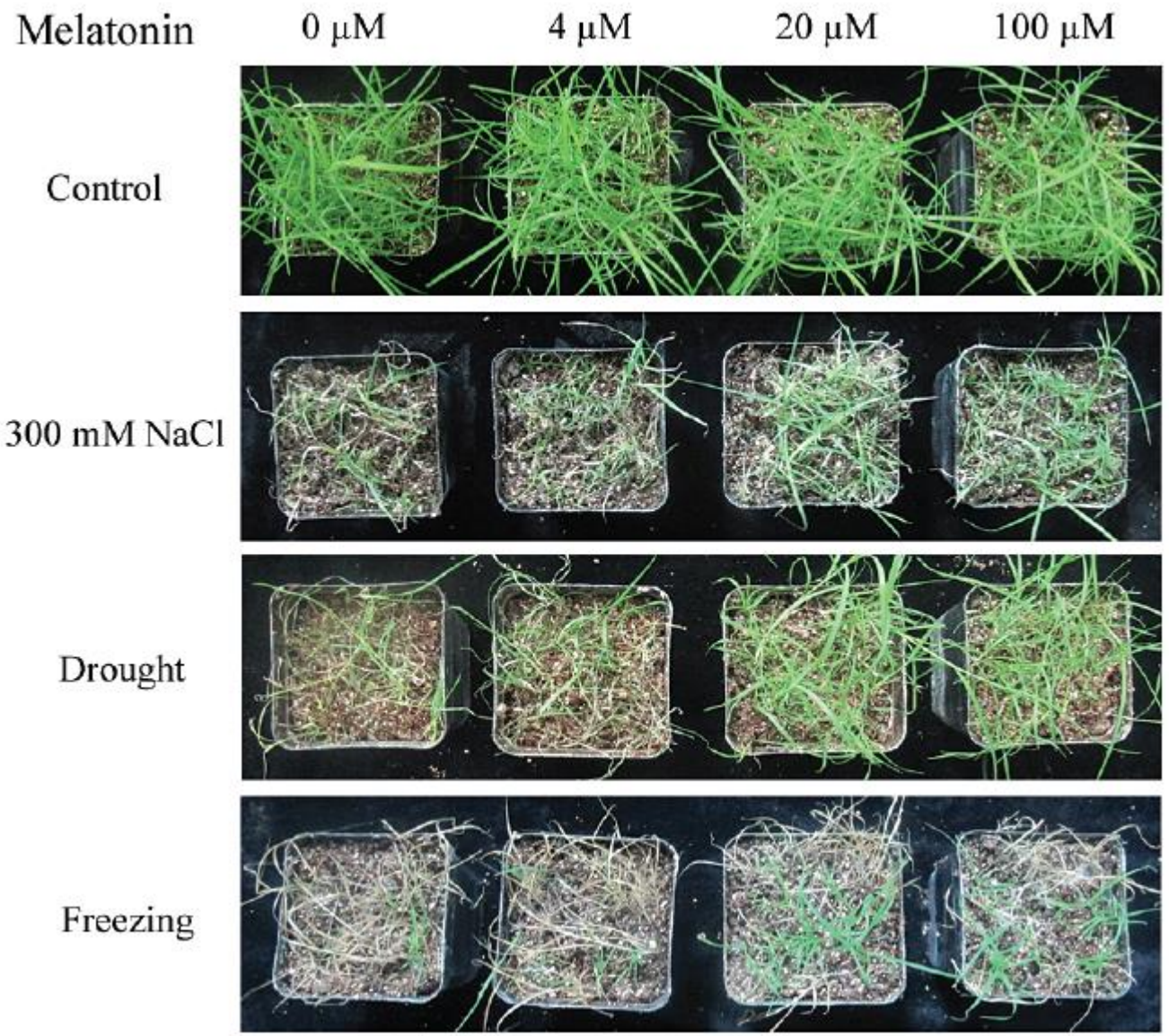
	Melatonin Content of Roots (ng · g FW^{-1})(± SE, n=5)
Water Stress	
Three irrigation (150 mL)	13.9 ±0.8
Two irrigation (100 mL)	39.4 ±2.6
One irrigation (50 mL)	56.8 ±3.3

Exogenous melatonin exposure for 3 days

Exogenous Melatonin Effect	
Control	7.7 ±0.5
Melatonin 1 µM	37.3 ±2.5
Melatonin 5 µM	58.9 ±3.4
Melatonin 10 µM	180.0 ±12.3

Arnao, 2014

Melatonin protects burmudagrass (*Cynodon dactylon*) from multiple stressors



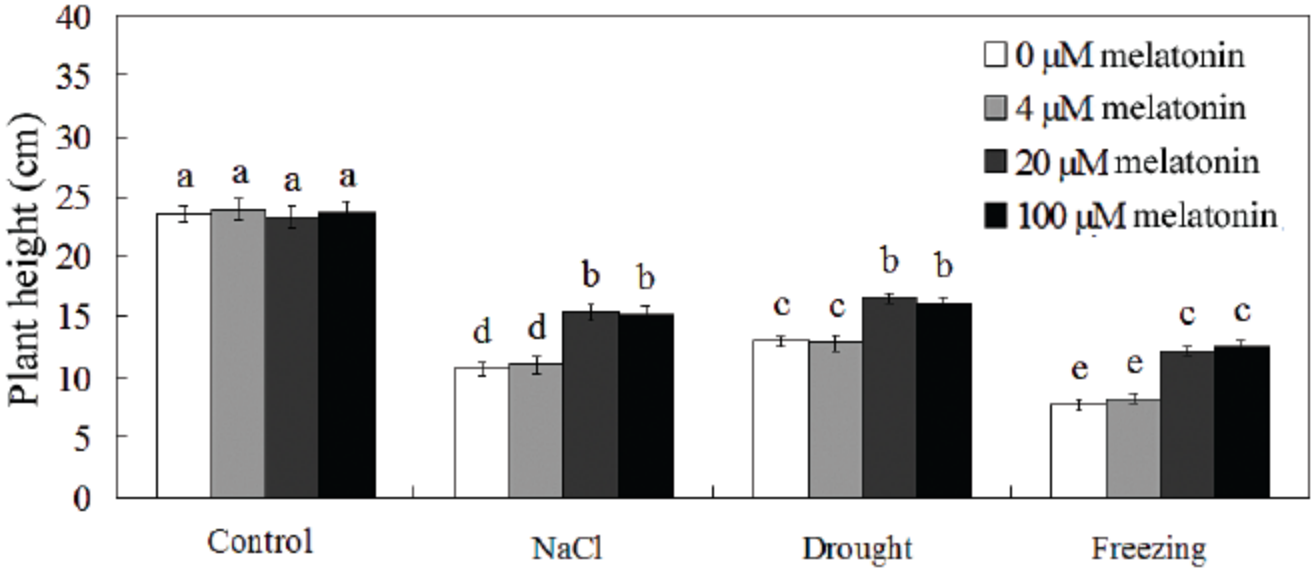
(Initially irrigated with water containing melatonin for 7 days)

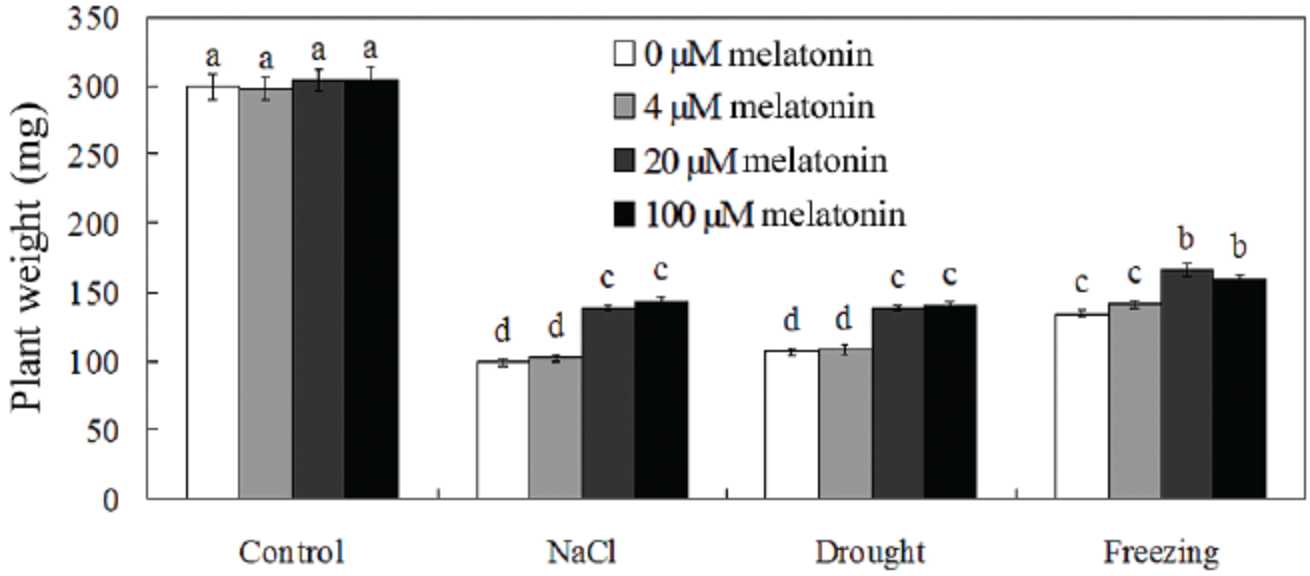
Survival improved
Chlorophyll preserved
Electrolyte leakage reduced
Greater plant height
Greater plant weight
ROS diminished
MDA reduced

Implication is that plants could be grown in harsher environments if melatonin enriched)

Shi et al, 2015

Exogenous melatonin protects bermudagrass from abiotic stresses

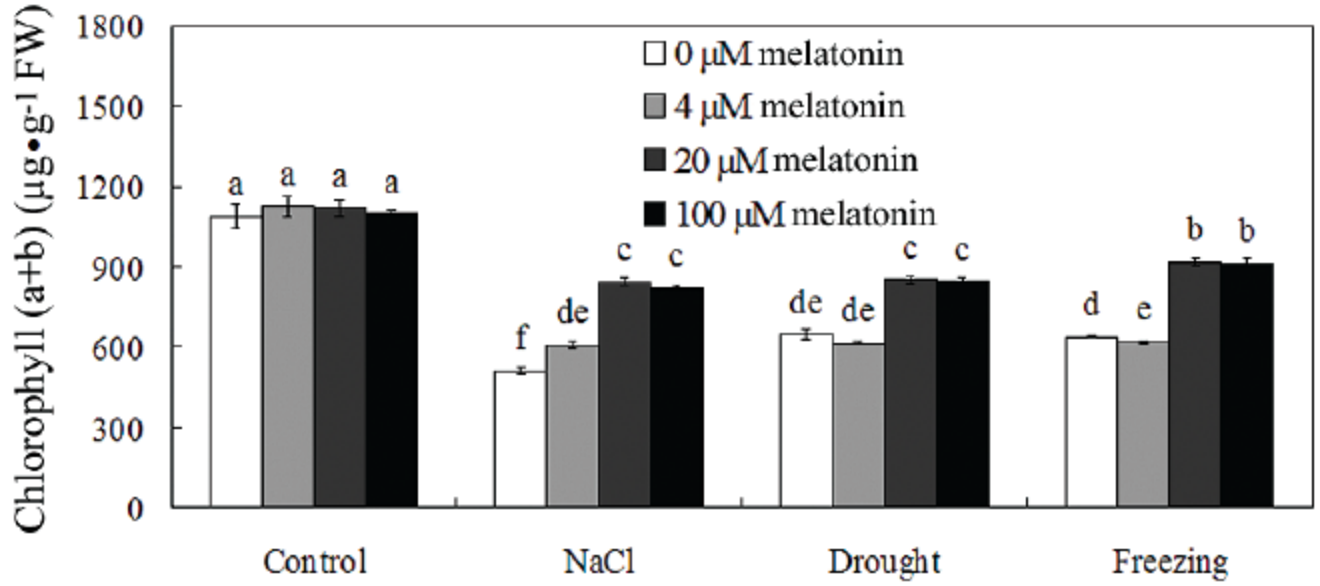


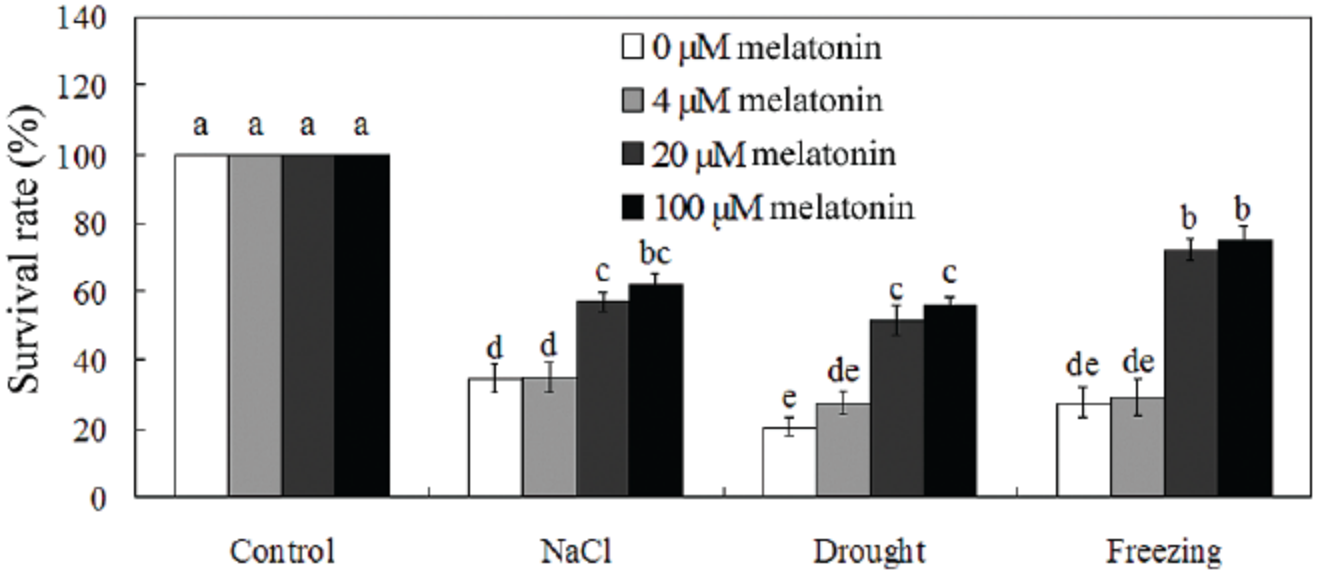


Melatonin Improves plant growth

Shi, Reiter, Chan, 2015

Exogenous melatonin protects burmudagrass fro abiotic stresses

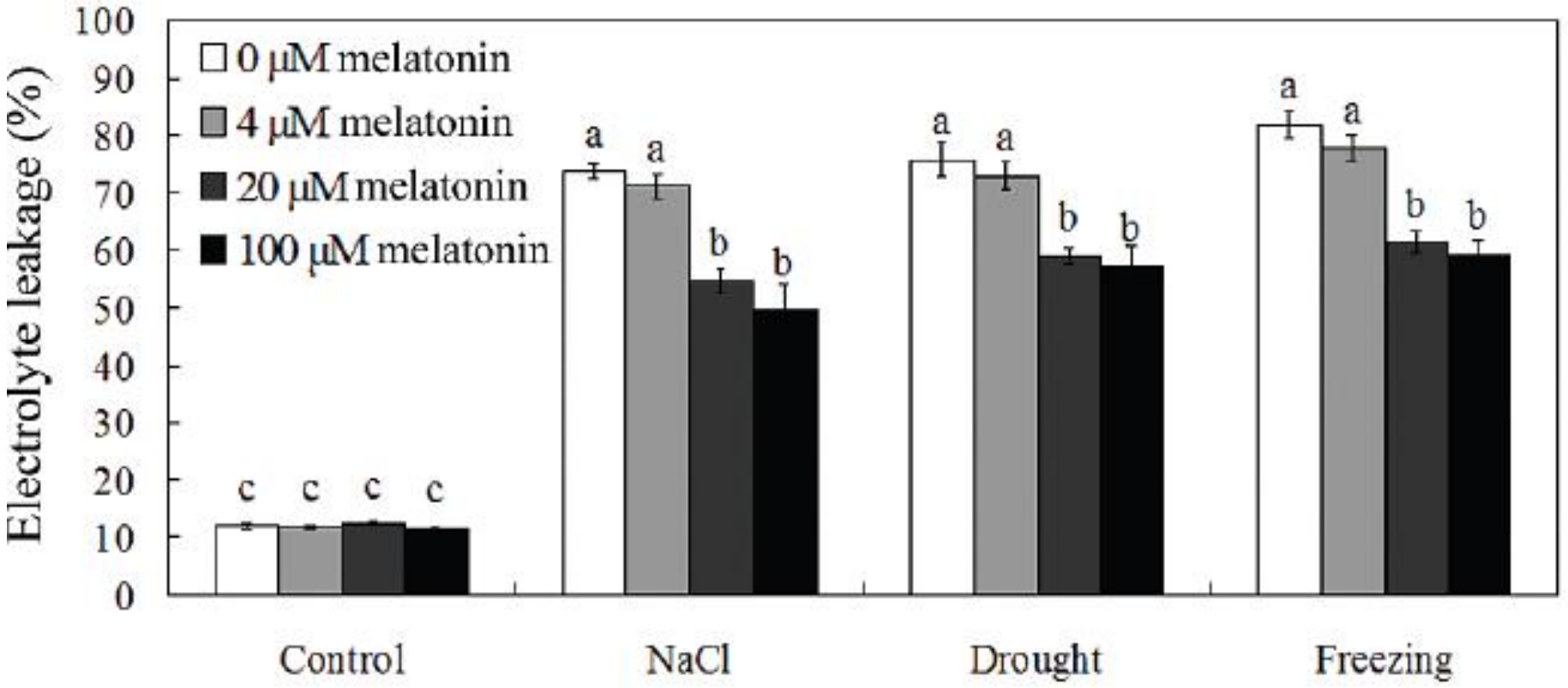




Melatonin protects chlorophyll and improves survival

Shi. Reiter, Chan, 2015

Exogenous melatonin protects burmudagrass from abiotic stresses



Melatonin reduces electrolyte leakage

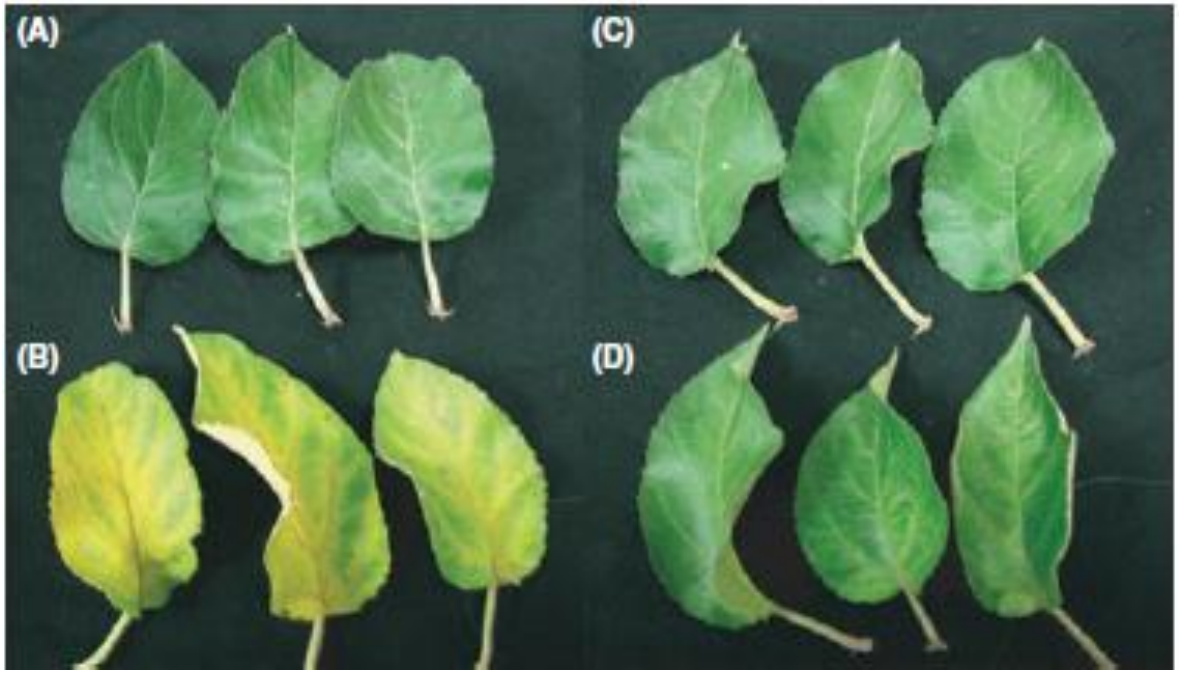
Shi, Reiter, Chan, 2015

Melatonin as an antioxidant in plants

Melatonin prevents Hanfu apple leaf yellowing after 90 days of drought

Watered control

Drought control



Watered w/ melatonin*

Drought w/ melatonin*

*100 µM to soil

Melatonin reduced 1), glutathione loss, 2), ascorbic acid reduction
3), leaf senescence (Sag12 gene) 4), reduced chlorophyll degradation (PAO gene)

Wang et al, 2013

Melatonin delays senescence of detached apple leaves

(*Malus domesticus*)



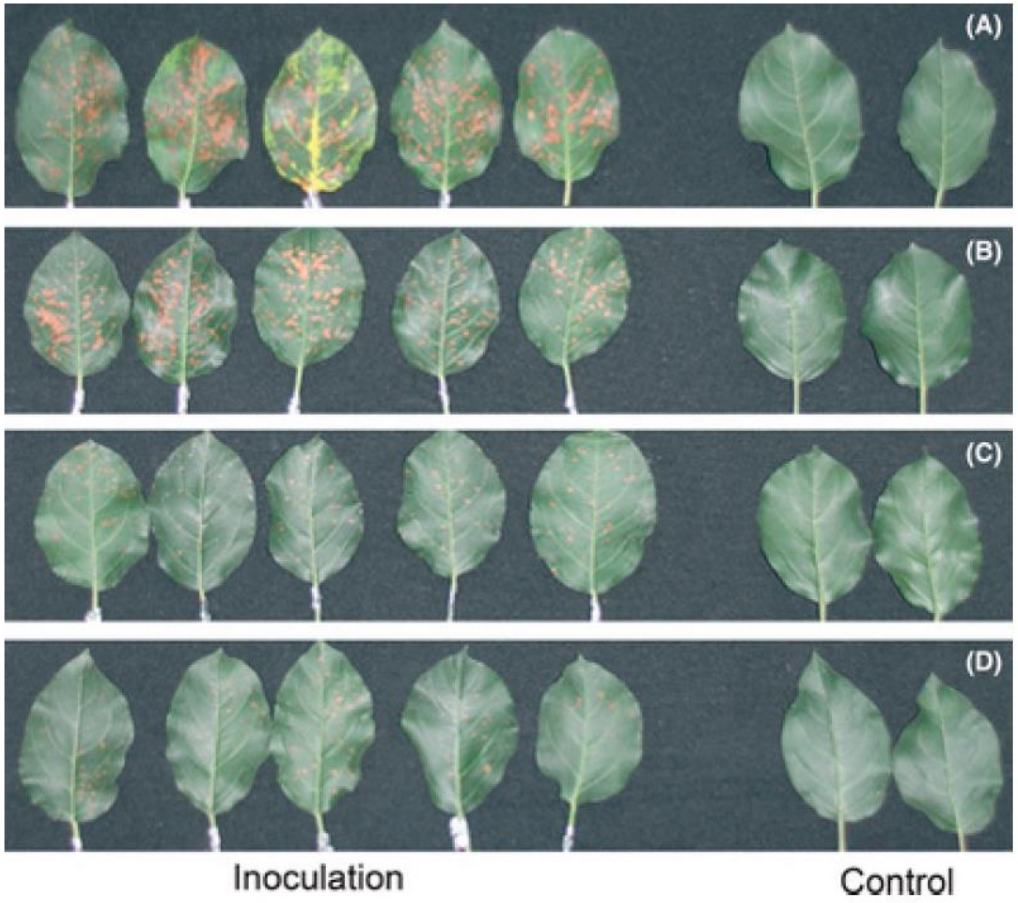
Maintained in dark for 12 days w/ or w/o melatonin

Melatonin

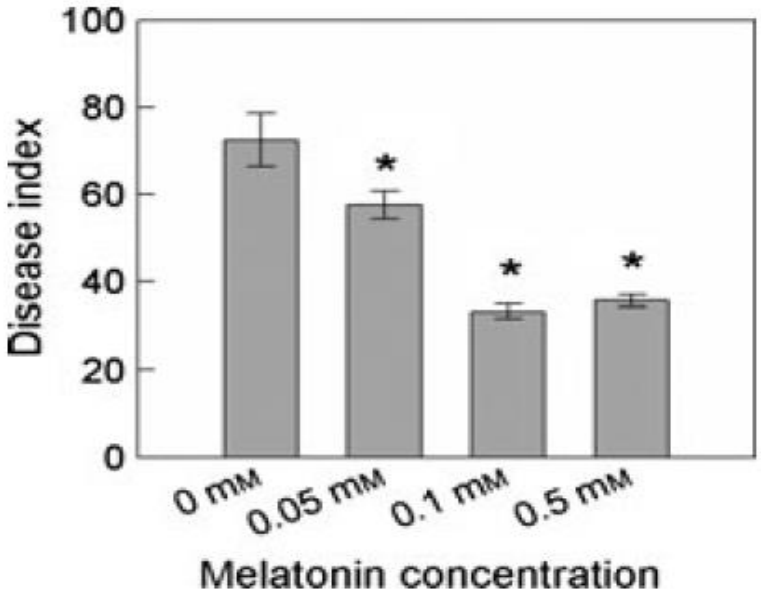
1. **Reduced chlorophyll loss**
2. **Suppressed *pheide a oxygenase***
3. **Suppressed *senescence-associated gene 12 (SAG12)***
4. **Maintained ascorbic acid**
5. **Maintained glutathione**

Wang et al, 2011

Melatonin protects apple leaves from biotic stress (apple blotch disease)



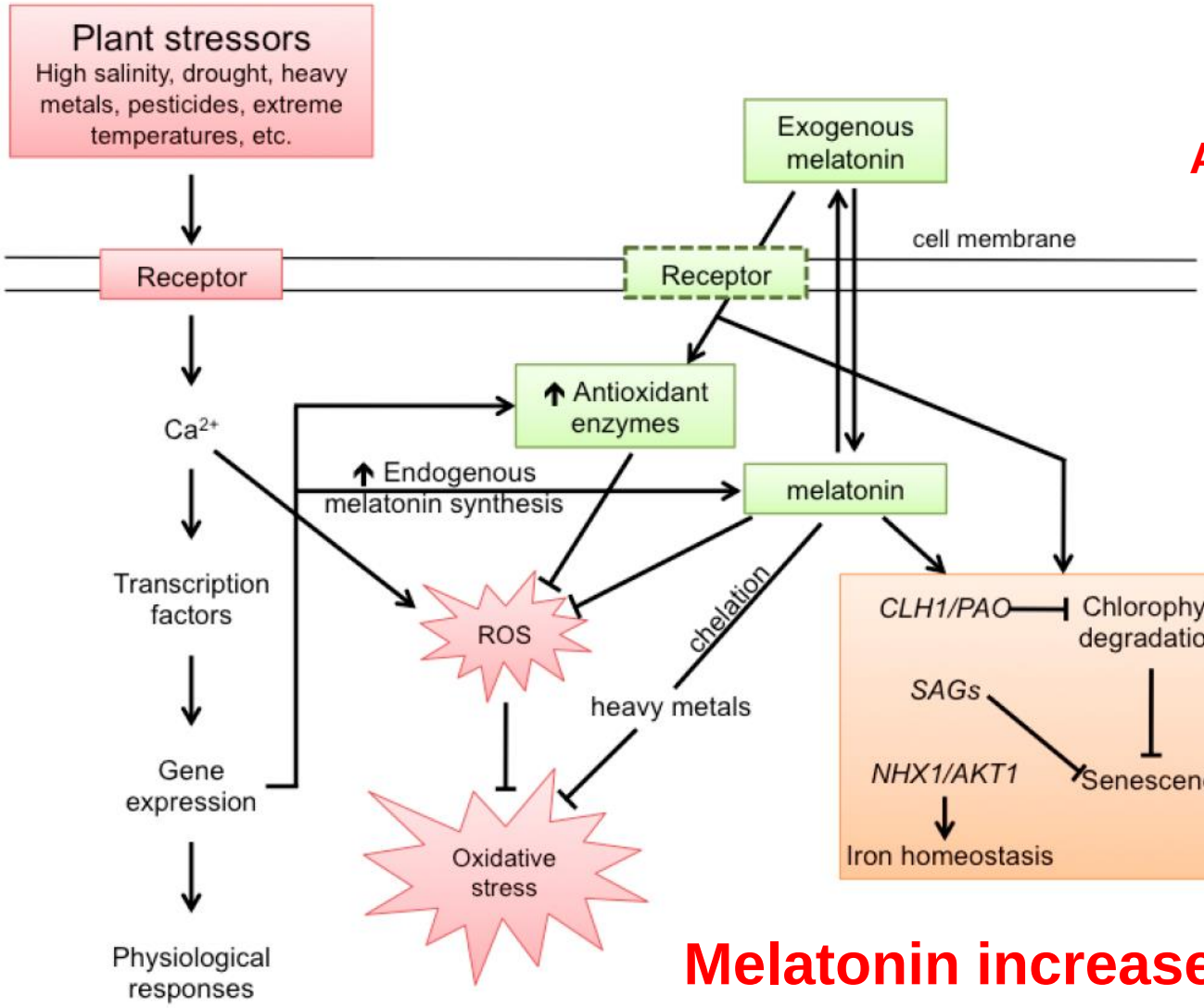
Plants irrigated for 3 days w/-w/o melatonin; brushed with Marssonia fungus spores



Melatonin improves plant resistance to fungus

Yin et al, 2013

Abiotic and biotic stressors induce melatonin synthesis in plants



Reiter, 2015

Abiotic stressors

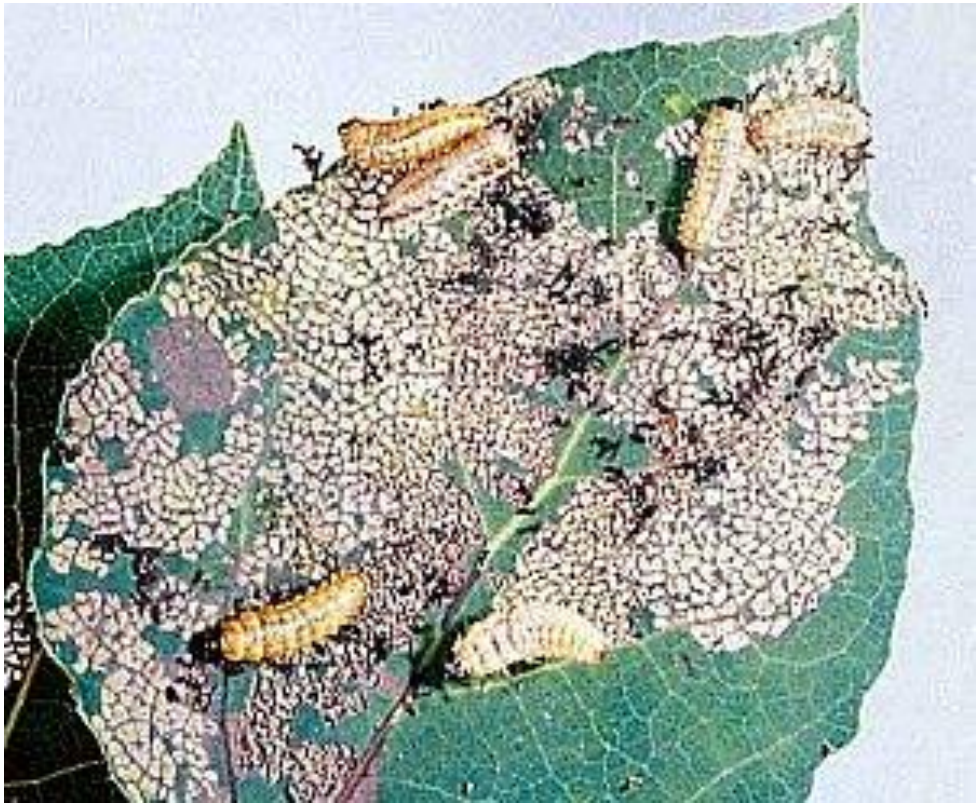
- High salinity
- Drought
- Extreme temperatures
- Pesticides
- Excess water
- Poor soil quality
- Heavy metals (cadmium/copper)

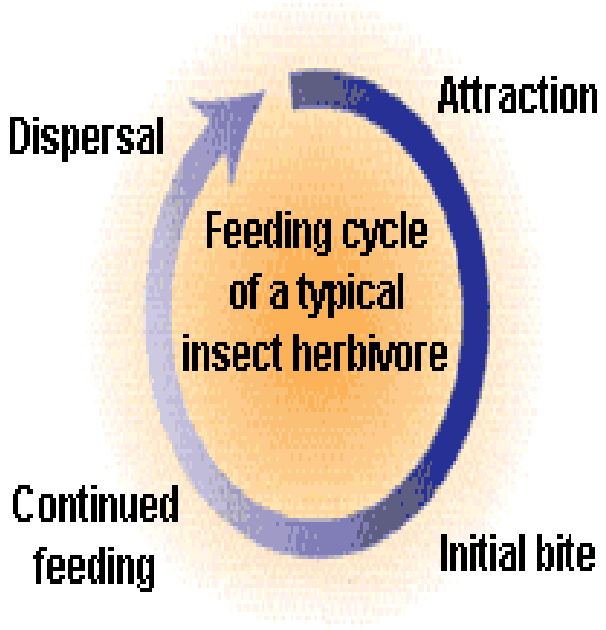
Biotic stressors

- Apple blotch
- Fungus
- Insects

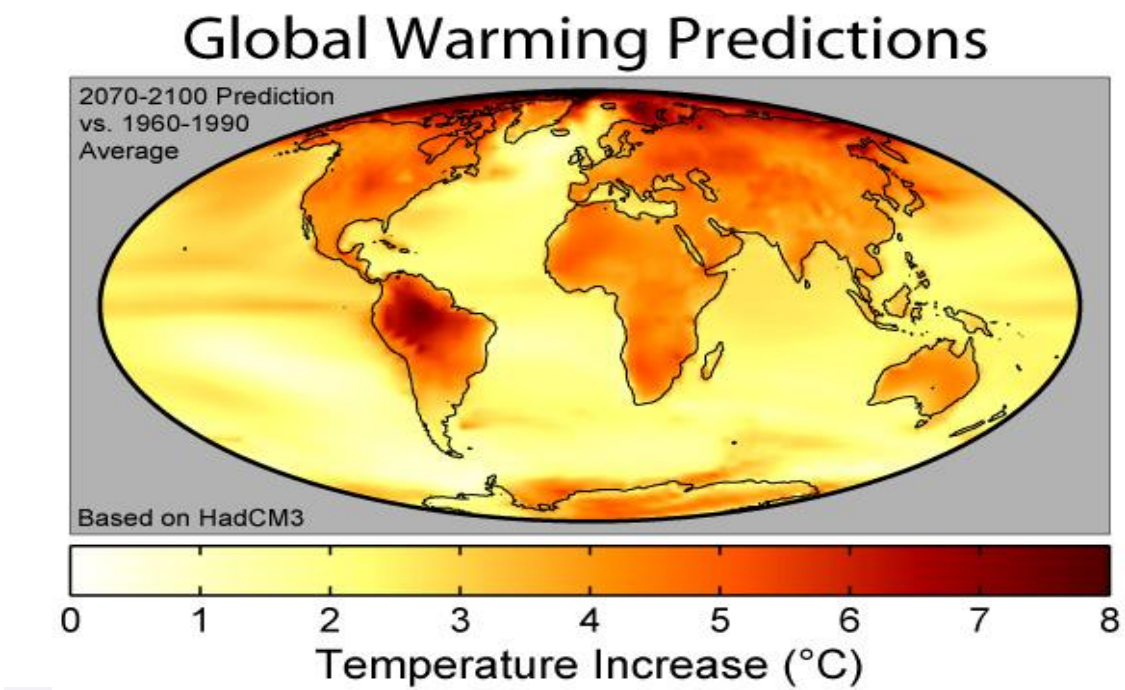
Melatonin increases the tolerance of plants to abiotic/biotic stresses

Does plant melatonin influence insect herbivores, e.g., leaf miners, stem borers, etc.?

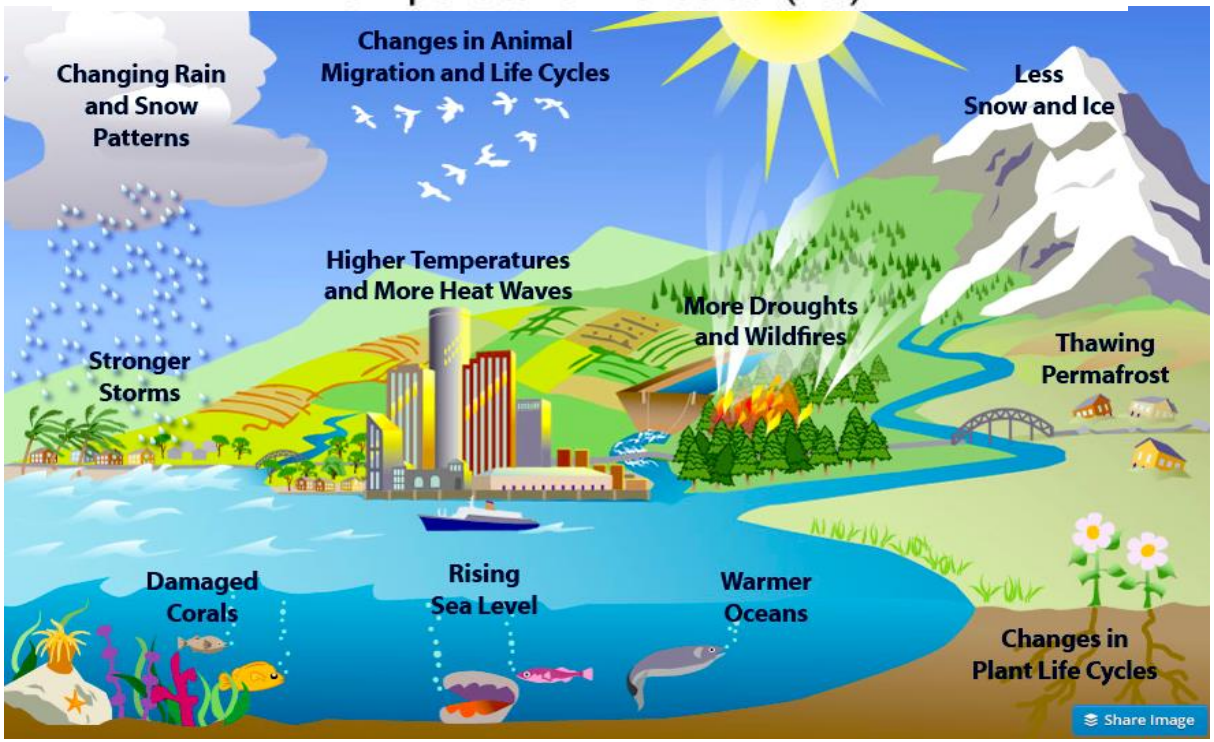




Billions of dollars of plant product are lost annually to insects



Impact of global climate change on plant growth and food production



Higher temperatures
More/longer droughts
Stronger rain storms
Changing plant life cycles
Warmer oceans

Conclusions

Melatonin helps plants increase tolerance to environmental insults

