



# Assessing Pesticide Tank Mix Compatibility And Combined Toxicity Effects On Monarch Butterfly Larvae

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### Interaction Disruption

Climate change is affecting ranges globally. Here ants are invading and consuming wildlife in cloud forest never before exposed to these marauders.

### Fire

Global warming elevates fire risk. Fires in Australia, Amazonia, and California burned an unprecedented >5 million hectares of forest in 2019.

### Storm Intensity

Climate changes bring stronger, more frequent storms and hurricanes; more fire-igniting lightening; and damaging flooding.

### Global Warming

Arctic sea ice is declining precipitously, arctic-alpine and other cold-adapted communities are contracting, while sea-level rise threatens coastal ecosystems.

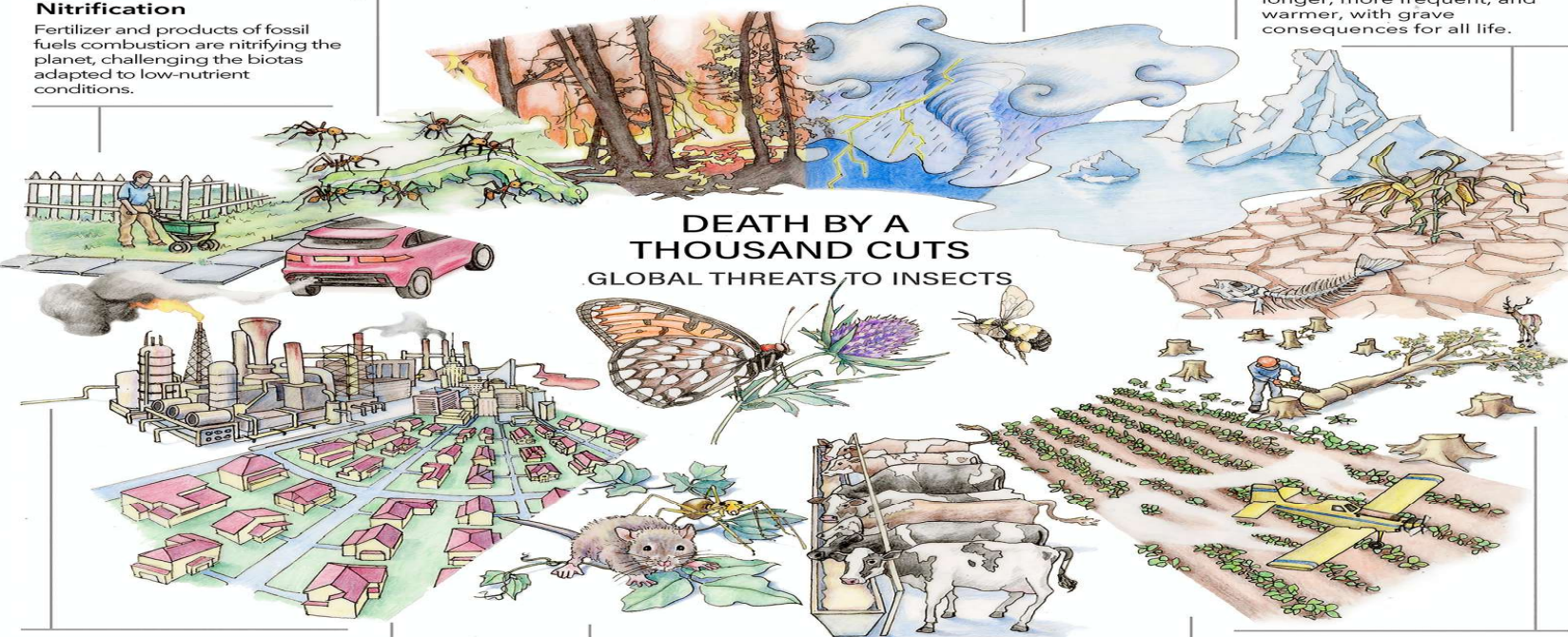
### Droughts

Periods with diminished precipitation are becoming longer, more frequent, and warmer, with grave consequences for all life.

### Nitrification

Fertilizer and products of fossil fuels combustion are nitrifying the planet, challenging the biotas adapted to low-nutrient conditions.

## DEATH BY A THOUSAND CUTS GLOBAL THREATS TO INSECTS



### Pollution

Chemical, light, and sound pollution of water, air, and soil are impacting plant and animal life worldwide.

### Urbanization

Our global population of 7.8 billion, spread planet-wide, comes at great cost to biodiversity and wildlands. Already, over 500 vertebrates have been driven to extinction.

### Introduced Species

Global trade is accelerating the movement of pernicious plants, animals, and pathogens to new regions—often with devastating consequences.



### Agricultural Intensification

Industrialized agriculture, with its attendant increases in scale, monoculturalization, nutrient input, and pesticide use, is becoming increasingly nature unfriendly.

### Deforestation

The tropics lost 11.9 million hectares of forest in 2019, mostly to agriculture.

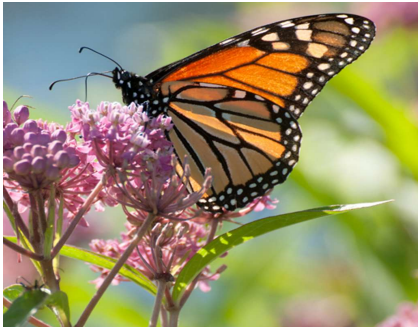
### Insecticides

Modern, industrialized agriculture, with its increasing reliance on chemical insecticides, has led to chronic contamination of wildlands and impacts to non-target insects.

Wagner et al. 2021

# Background

- ▶ In 2022, Environmental Protection Agency announced assessing risk of pesticides on listed species and their critical habitat.
- ▶ 99 Federally listed insect species
  - ▶ Over 40% Lepidopterans



**Monarch butterfly**



**Blue butterfly**



JOURNAL ARTICLE

# Monarch Butterfly Ecology, Behavior, and Vulnerabilities in North Central United States Agricultural Landscapes

Tyler J Grant, Kelsey E Fisher, Niranjana Krishnan, Alexander N Mullins, Richard L Hellmich, Thomas W Sappington, James S Adelman, Joel R Coats, Robert G Hartzler, John M Pleasants ... [Show more](#)

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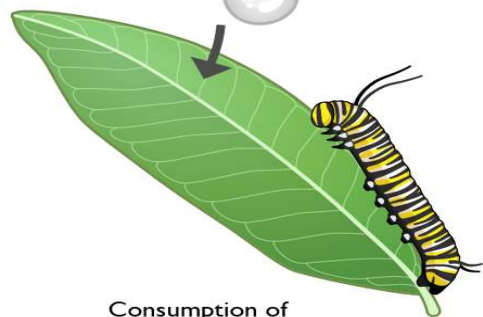
# Objectives

- ▶ Evaluate the compatibility of biological fungicides and conventional fungicide in tank mixes
- ▶ Assess the individual and combined pesticide toxicity effects on monarch larvae and eastern tailed-blue larvae

# Pesticide Exposure Routes

## Dietary

Pesticide absorbed into leaf tissues



Consumption of pesticide by larva

Pesticide incorporated into nectar



Consumption of pesticide by adult

## Topical



Larva or adult exposed to airborne pesticides



Larva exposed to pesticide residue on leaves



# Pesticides Used

## Formulated Products:

### Biological Fungicides:

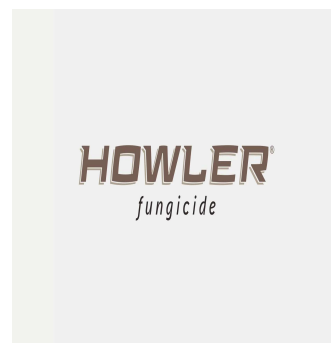
- ▶ Double Nickel® (*Bacillus amyloliquefaciens*)
- ▶ Howler® (*Pseudomonas chlororaphis*)

### Chemical Fungicide:

- ▶ Kocide® (Copper-based fungicide)

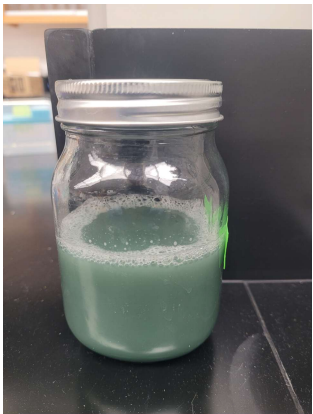
### Chemical Insecticide:

- ▶ Bifenture (Pyrethroid)

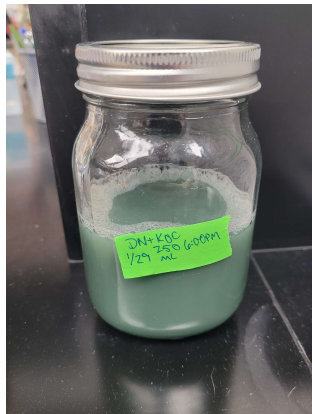


# Compatibility Testing

- ▶ Combinations at tank mix rate;
  - ▶ Double Nickel<sup>®</sup> + Kocide<sup>®</sup>
  - ▶ Howler<sup>®</sup> + Kocide<sup>®</sup>



DN + Ko at 24 hr



DN + Ko at 48 hr

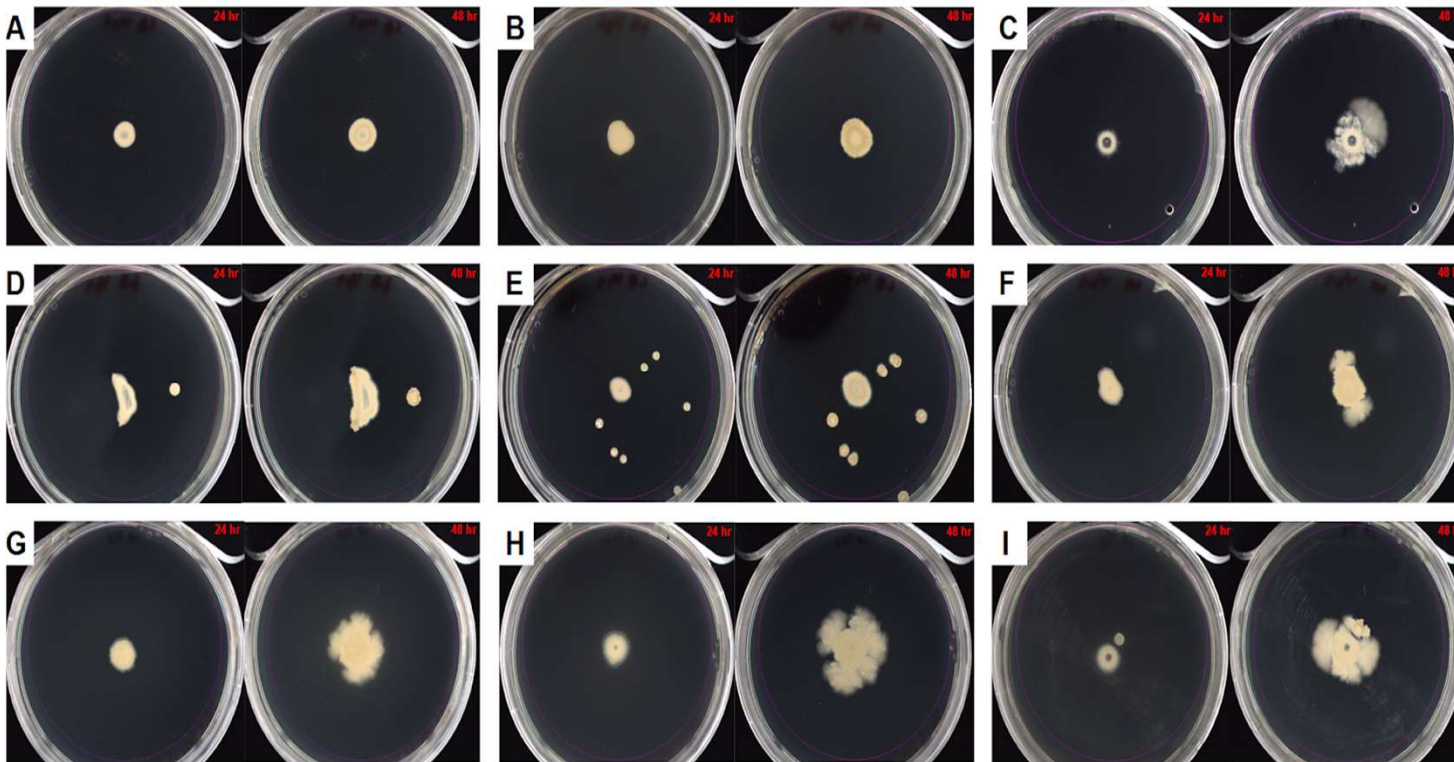


HW + Ko at 0 hr



Sabrina

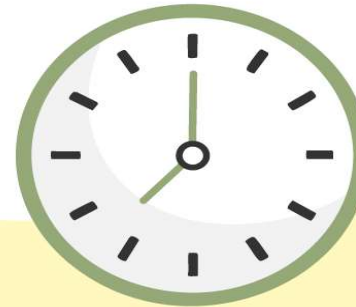
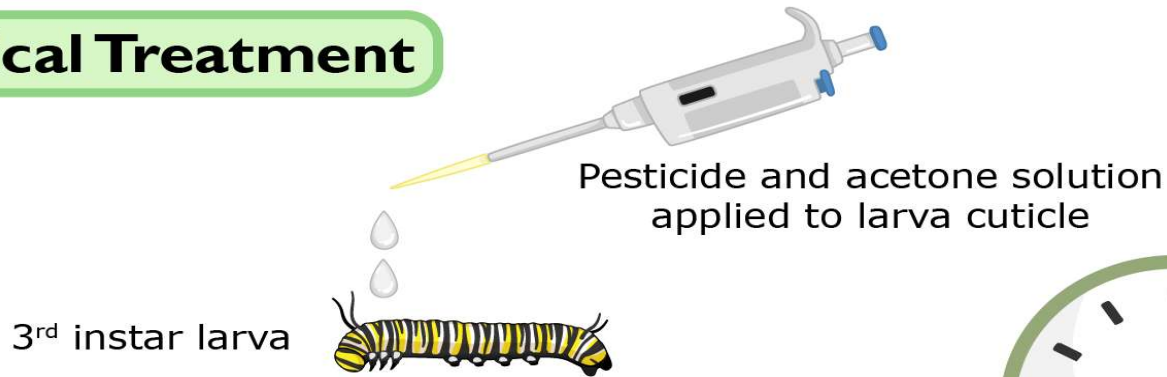
# Plating



A-C = Double Nickel® only  
D-F = Double Nickel® + Kocide® at 0hr  
G-H = Double Nickel® + Kocide® after 24hr

# Materials and Methods: Acute Toxicity Bioassays

## Topical Treatment



## Dietary Treatment



Observations taken every 24hr for 4 days or until pupation

# Individual Toxicity

Treatment concentration was the maximum application rate

Name	Exposure	Maximum application rate concentrations	#larvae treated	% larval mortality
Double Nickel®	Cuticular	30 µL/mL	30	0
	Dietary	1.5 µL/g	30	0
Howler®	Cuticular	18 µg/uL	30	0
	Dietary	900 µg/g	30	13
Kocide®	Cuticular	3.6 µg/uL	30	0
	Dietary	180 µg/g	30	0

**Bifenture caused 100% mortality in both bioassays**

# Combined Toxicity

No mortality at the maximum application rate

Name	Exposure	#larvae treatment	%larval mortality
Double Nickel® + Kocide®	Topical	30	0
	Dietary	30	0
Howler® + Kocide®	Topical	30	0
	Dietary	30	0

## In summary

- ▶ Double Nickel<sup>®</sup> , and Howler<sup>®</sup> are physically compatible with the copper hydroxide in Kocide<sup>®</sup> at maximum application rates
- ▶ Double Nickel<sup>®</sup> is biologically compatible with Kocide<sup>®</sup> at the maximum application rates
- ▶ Double Nickel<sup>®</sup> , Howler<sup>®</sup> pose *de minimus* risk to monarchs at maximum application rate.

## Future Plans

- ▶ Eastern-tailed blue (Lycaenid) toxicity studies (surrogate for listed lycaenids)

# Acknowledgement



Krishnan lab members

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