Maira Bonini (IT)	Introduction of the course
Michel Thibaudon (FR)	Allergy to ragweed and allergens involved
Chiara Montagnani (IT)	How to recognize the different *Ambrosia* species
Branko Sikoparija (SRB)	How to distinguish *Ambrosia* pollen from other similar pollen
Rea Maria Hall (CH)	**How to manage the *Ambrosia* plants**
Heinz Müller Schärer & Carine Beuchat (CH)	*Ophraella communa*: biology, impact, biosafety and recognition

Ragweed
(Ambrosia artemisiifolia L.)

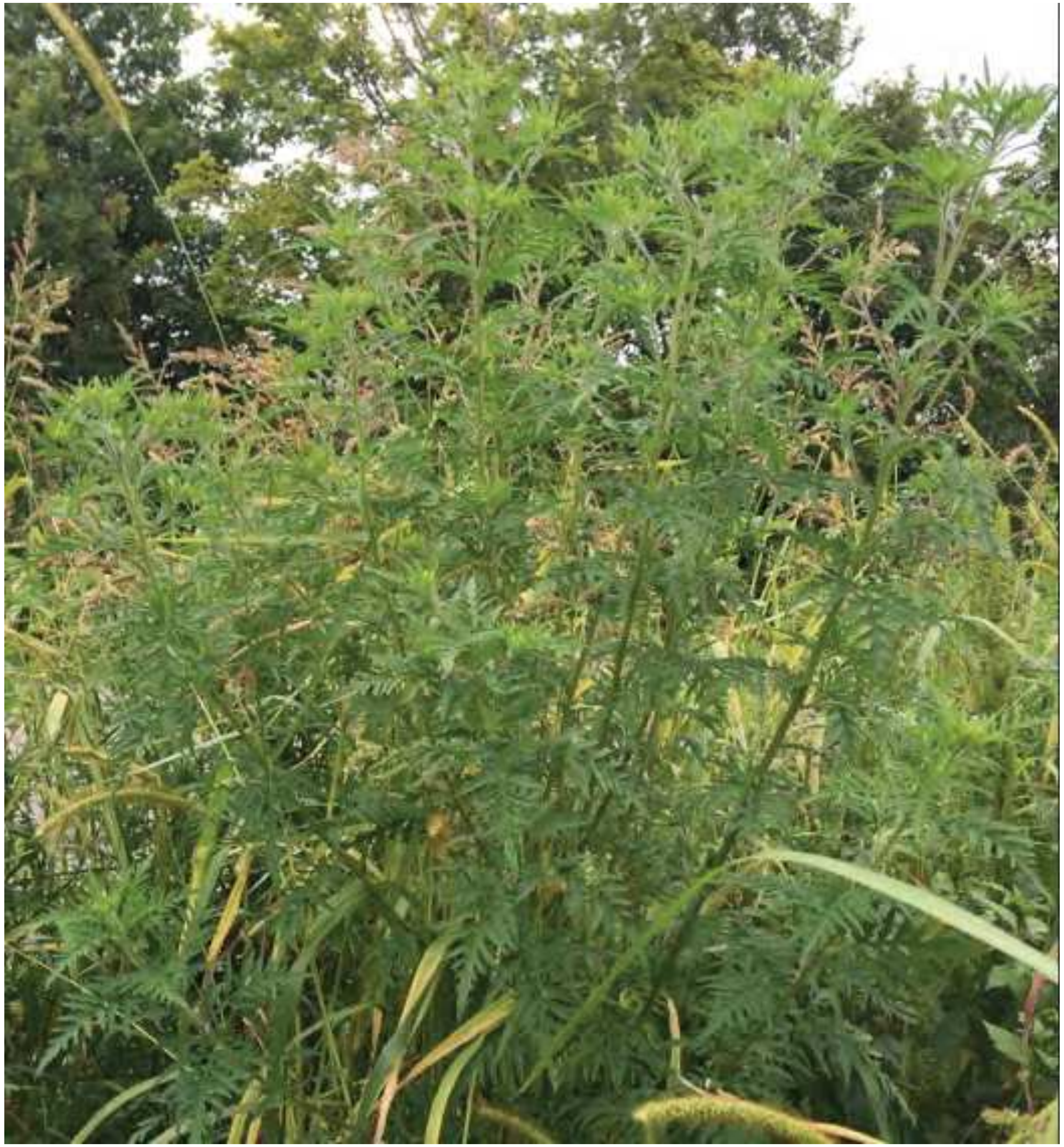
The weed, that came to stay!

Rea Maria Hall
University of Natural Resources and Life Science Vienna

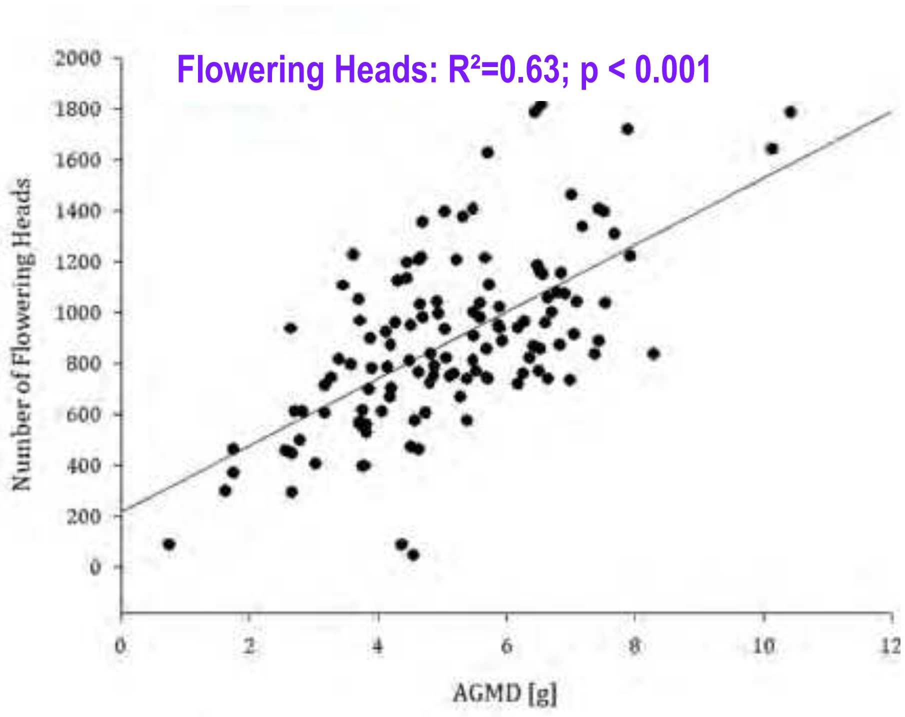
Ambrosia artemisiifolia L.
Common ragweed

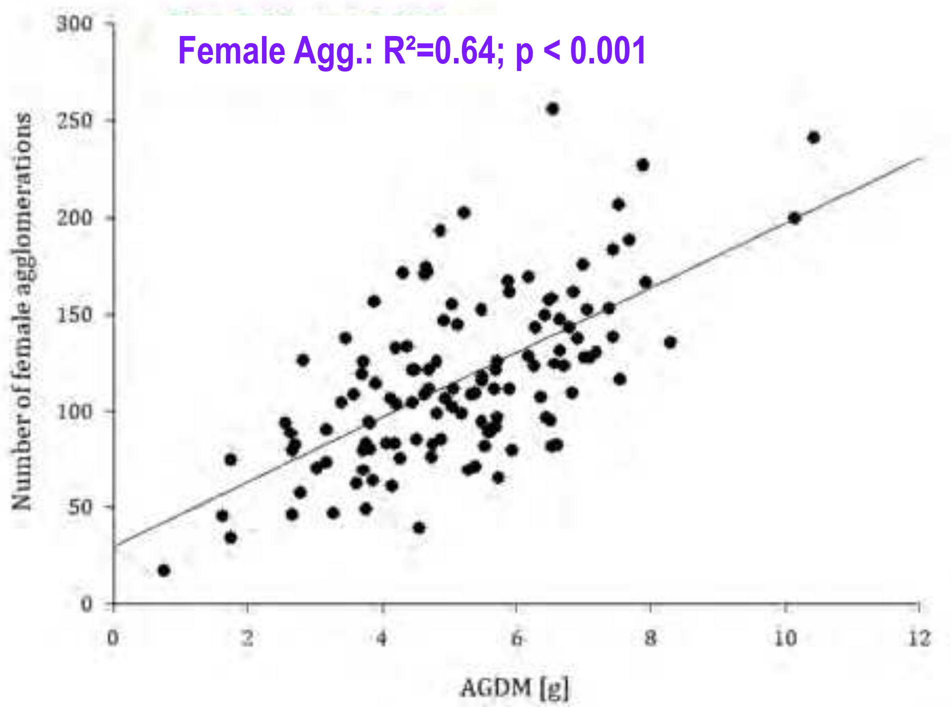

Biology

- **Family:** Asteraceae
- **Life Span:** summer annual
- **Photoperiode:** shortening-day
- **Germination:** late spring – to early summer
- **Growth height:** up to 2.5 m (highly plastic)
- **Growth rate in first month:** up to 20 mg d^{-1} (soybean: 8-12 mg d^{-1})
- **Male inflorescence:** mid of July
- **Main flowering season:** August – September→ up to 8 bn. pollen/plant
- **Competitive power:** medium but very high plasticity → plants can adapt to almost all environmental conditions



More biomass = more pollen and seeds





- **Per gramm biomass dry matter 177 flowering heads are developed on average**
- **Per gramm biomass dry matter 23 female agglomerations are developed which contain 5 to 20 seeds on average**

Development steps

week 1

week 2

week 3

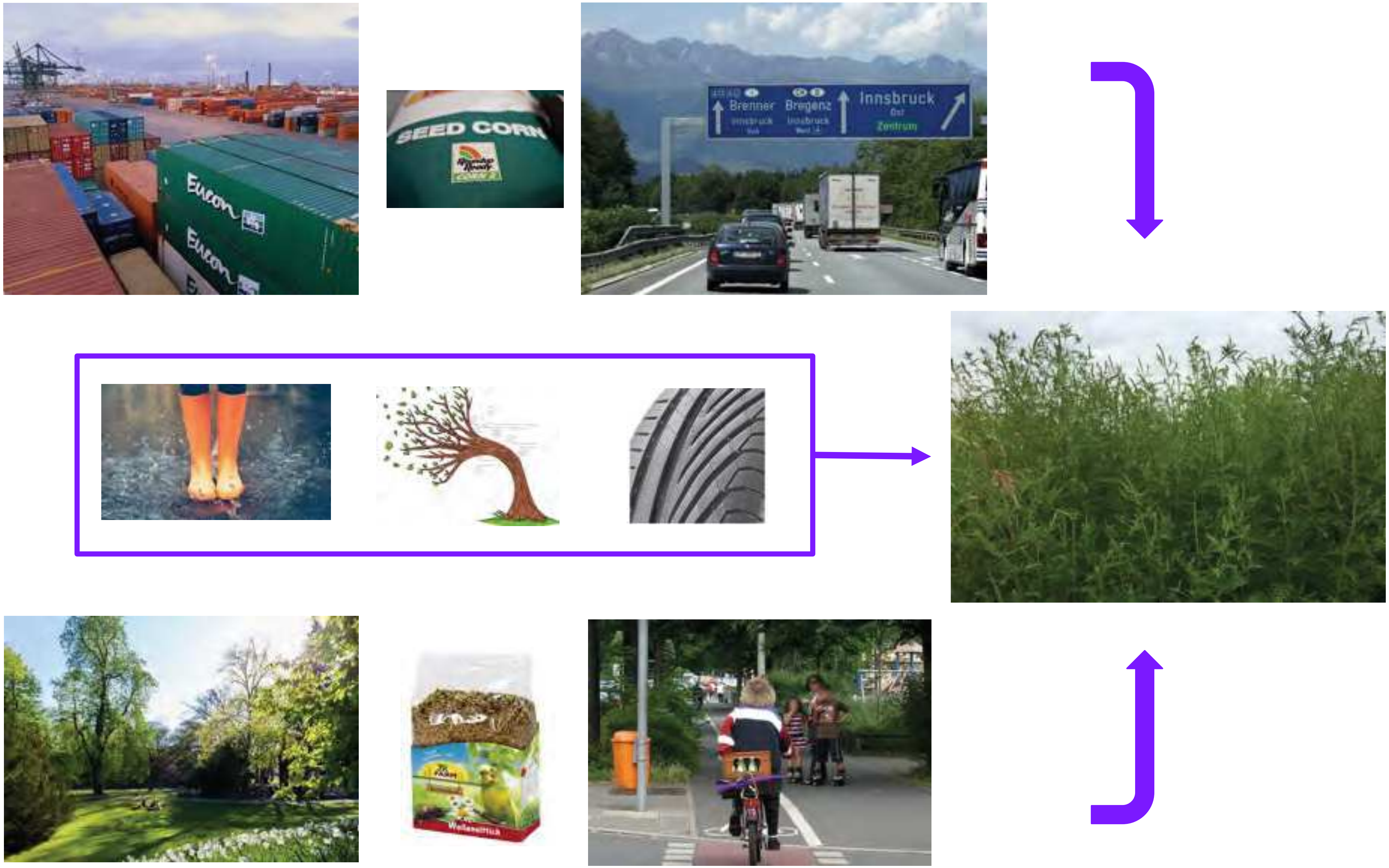
week 4

week 5

week 6

week 11

Distribution pathways



Spread across Europe and Austria

- First appearance of the plant in Western Europe was documented in Pfaffenberg (Brandenburg, Germany) in the year 1863
- Main naturalization and establishment of the plant in Europe occurs after WW II (Kazinczi et al., 2008)
- The geographical route of distribution can be traced back to harbors like Rijeka, Trieste and Genoa (Comtois, 1998)
- In Austria, a steady spread of the plant was observed since the 1960ies, primarily along the high-capacity road system (Karrer, 2007; Kazinczi et al., 2008)
- Particularly, agricultural machinery and implements of the road maintenance services contribute substantially to the further transmission of the seeds (Vitalos und Karrer, 2009)
- Natural distribution along riverruns and through soil movement

Economic impact

- Infestation of crop fields with ragweed can cause harvest losses between 20 and 70 %, depending on the crop
  (Bullock et al., 2010; Novak et al., 2009)
- Yield losses in oil seed pumpkin can rise up to 70 % (Kazinczi et al., 2008)
- 3 plants per squaremetre decrease photosynthesis performance of soybean by approx. 30 % (Coble et al., 1981)
- Proteins in the ragweed pollen have strong allergenic effects. They were identified to be a significant cause of allergic rhinitis and hay fever during late summer and autum.
- In Austria, annual cost per person concered are estimated at 630 € >> in Austria approx. 1 mio. people are affected by ragweed pollen (LK Steiermark, s. a.)
  >> in 2016 social cost due to ragweed allergy exceeded the 100 Mio. € mark (Medical University Vienna)

The seeds = the factor of success

- **Fruit = achene,** covered by a dry spiny involucrum
- **Average seed production:** ø 1.000-3.000 seeds/plant (max. 62.000; Kazinczi et al., 2008; Fumanal et al., 2008)
- **Ripening:** from September onward
- Primary dormant seeds
- Stratification (4°C – 8 weeks; Willemsen & Rice, 1972)
- **Soil seed bank:** persistent >> seeds can remain germinable in soil up to 40 years (Toole & Brown, 1949)

- > several studies indicated that viability and vitality decrease constantly, even under constant storage conditions (Long et al., 2015; Ratajczak et al., 2015)

Burial trial

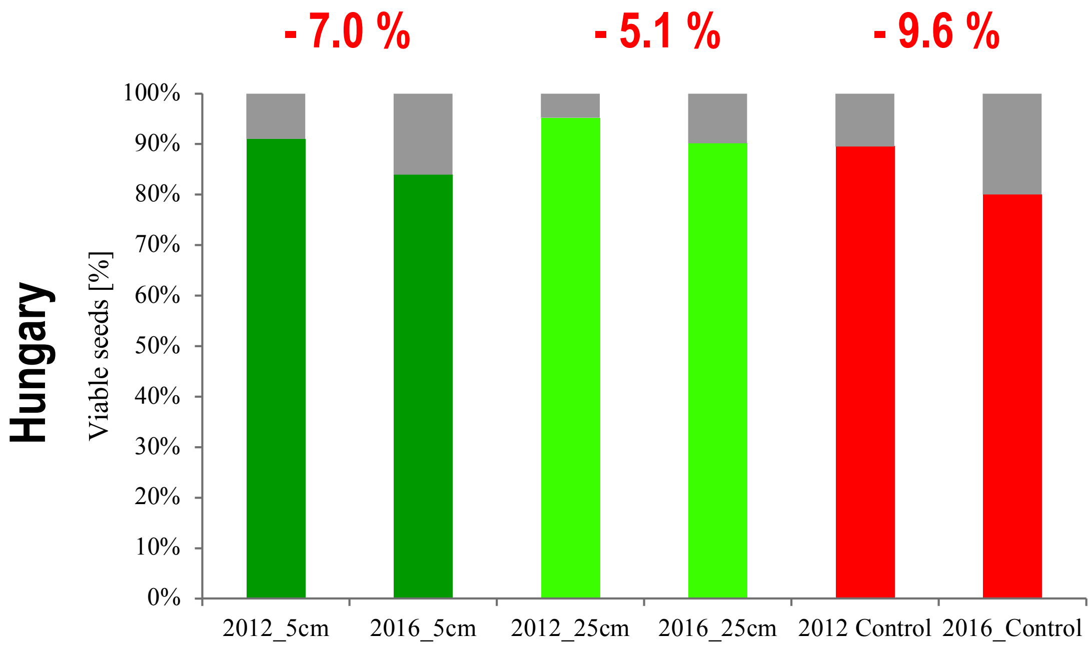
- Within the HALT Ambrosia project a long-term experiment was conducted to test seed longevity of ragweed seeds from 2 different origins in 2 different depths (5 and 25 cm)
- Seeds were enclosed at portions of 50 in fine polyethylene nets
- Since 2012, every year in early spring a randomly chosen subset of 5 nets per depth layer are digged out at all sites (until 2022)
- Intact seeds were counted and tested for viability by germination test and subsequent TTC-test

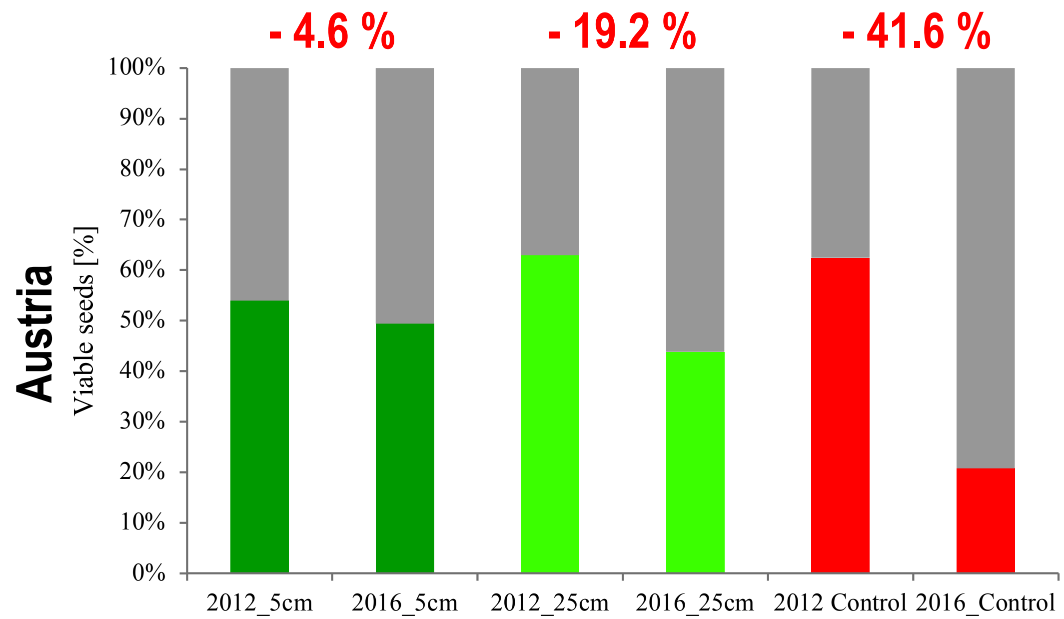
Main questions:

- Do seeds of common ragweed in lower or in upper soil layers decay faster?
- Do seeds of common ragweed decay faster in soil than under controlled conditions?

Burial depth

- Mean values of 3 different European labs which participated in this joint-trial
- With the Austrian population a significantly higher degradation rate was monitored in the lower soil layer (25 cm)
- No significant differences were detectable with the Hungarian seeds
- Highest degradation rate was observed under controlled storage (4°C in darkness)





Very high adaptive capacity

- Very high flexibility in terms of growing and environmental conditions
- **High tolerance against:** salt, heavy metals, nutrient deficite, drought and contaminants like tyre abrasion
- Different life cycle compared to other (native) plant species >> ragweed can evade common/typical management regimes
- actually existing management systems promote establishment, growth and spread of ragweed (i.e. mowing regimes along road verges)
- **Very high regenerative capacity after management intervention**

Further problems in controlling ragweed

- almost no effective herbicides particularly with crops like soybean, potatoe, different kinds of vegetables, sugar beet and grassland
- particularly along road verges and railways herbicide application is often not allowed due to environmental and human health issues
- very quick development of resistancies (4 years with glyphosate)
- on ruderal areas and low-interest-sites (dumps, landfill sites, hedges, timer stockyards, field paths etc.) an effecitive management would be completely uneconomic and unfeasable
- conflicts of competence

GOAL: Road sides

Development of a management system, which significantly reduces/prevents the further spread of ragweed

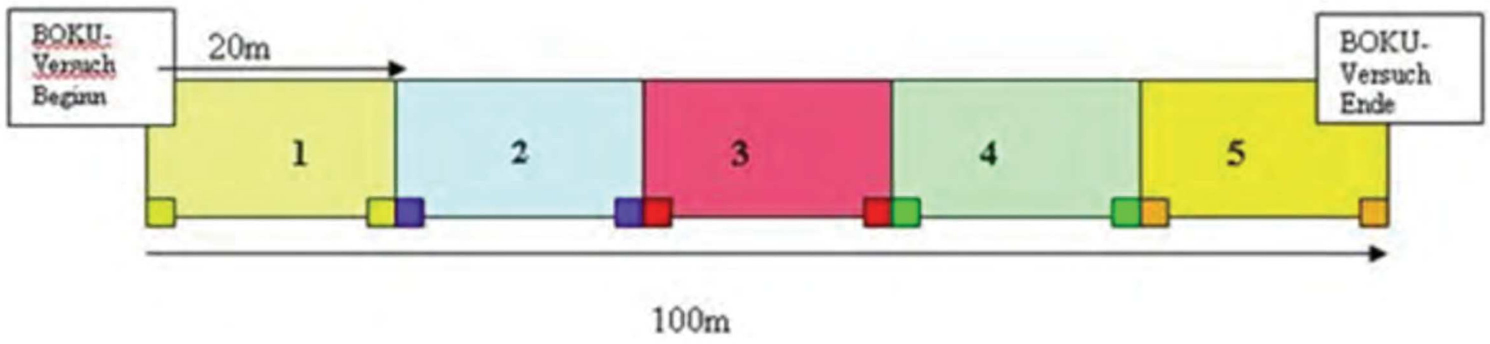
Status quo

The most common mowing regime in Austria and Germany

- **1. Cut (April/May):** competitive plants are removed/damaged
  >> ragweed can germinate freely
- **2. Cut (end of June):** kif ragweed has grown high enought (min. 5 mm) the plants are damaged instead of apical growth the plant switches to basal growth = more stems, more males more seeds
  - during summer native plants reduce their growth to a minimum, whereas ragweed continue to grow due its high tolerance against heat and drought
  - no cutting event during summer (holidays etc.) >> ragweed can develop pollen and seeds almost undisturbed
- **3. Cut (September/October):** ragweed seeds are ripe and are further spread by mowing- and mulching machinery (up to 90 seeds per 100 g biomass)
- **Further aspect:** road construction >> distribution of seeds with contaminated soil

Experiment to find the optimum cutting regime (2009-2011)

5 mowing regimes – 7 sites



Treatment 1: no mowing (control)

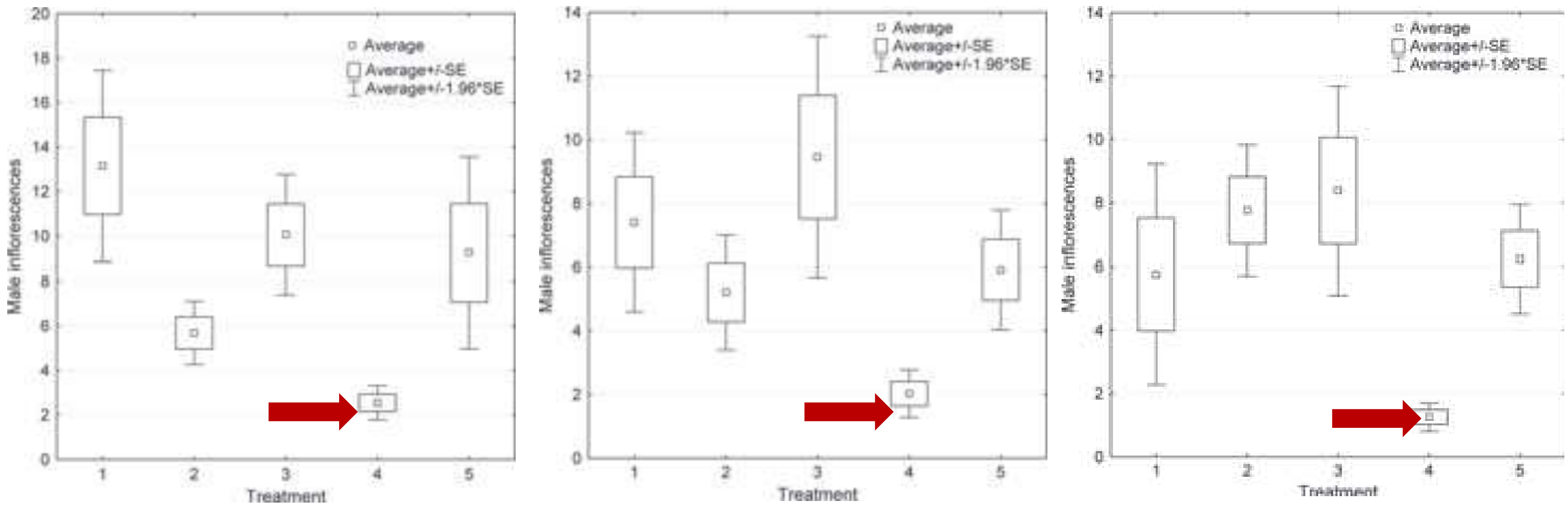
Treatment 2: 1. Cut: last week of June (before the start of the flowering)
2. Cut:: second week of September

Treatment 3: 1. Cut: third week of August (after the beginning of flowering)
2. Cut:: second week of September

Treatment 4: 1. Cut: last week of June (before the start of the flowering)
2. Cut: last week of July (before mass flowering)
3. Cut:: second week of September

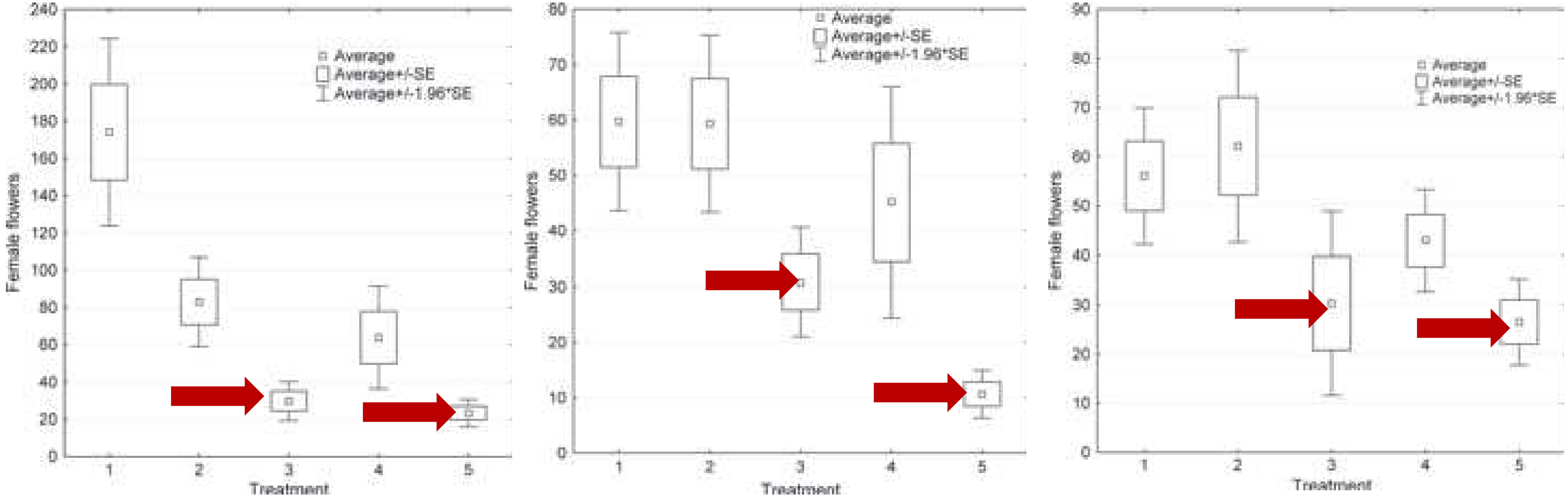
Treatment 5: 1. Cut: last week of June (before the start of the flowering)
2. Cut: third week of August (after the beginning of flowering)
3. Cut:: second week of September

Number of male flowers



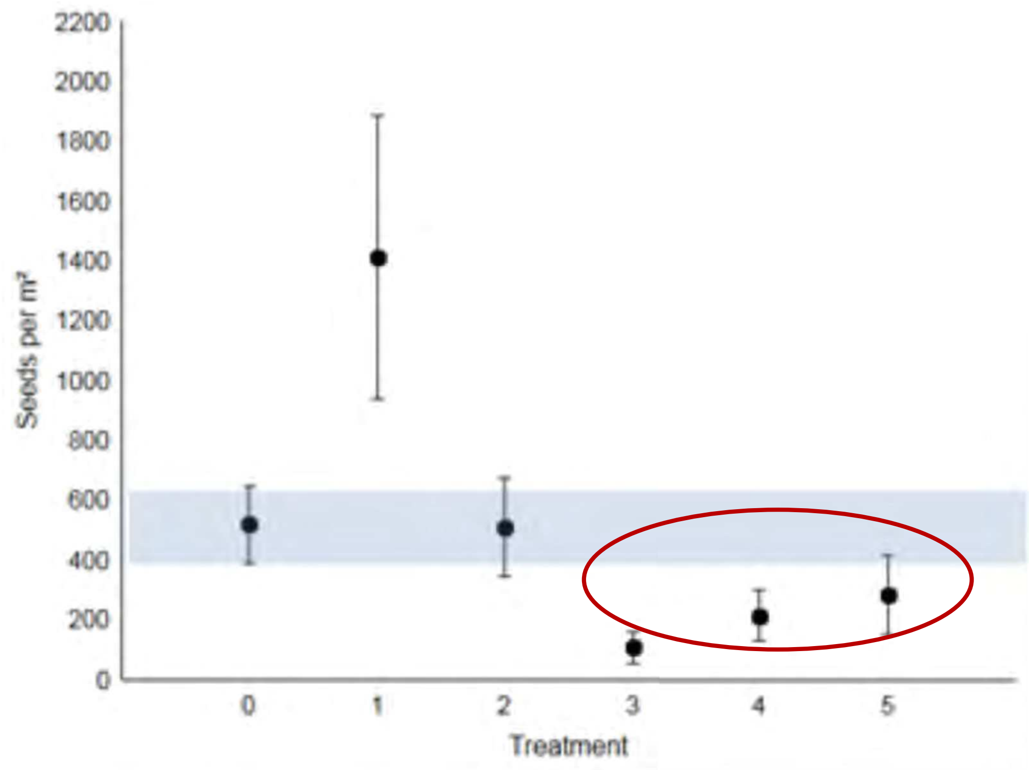
- During all 3 years, road verges under cutting treatment 4 showed the significantly lowest number of male flowers (= pollen)
- <u>**Treatment 4:**</u> last week of June / last week of July / second week of September

Seed production



- During all 3 years cutting treatment 3 and 5 were the best choice to reduce seed production
- **Treatment 3:** third week of August / second week of September
- **Treament 5:** last week of June / third week auf August / second week of September

Soil seed bank



- After these 3 years it became obvious that treatment 3, 4 and 5 are most suitable to deplete the soil seed bank

GOAL: Agriculture & Nature conservation

Development of crop rotation systems and farm routines which prevent the further spread of ragweed


One single seed contamination event is enough to infest a site (agricultural field, meadow etc.) sustainably.
Each seed which enters our fields today is a potential threat until the year 2038!!!

BFFs: sugar beet & ragweed

- Special dispersal dynamic in interaction with sugar beet
  - almost no negative effect on yield >> no special interest to eradicate the plant BUT: ragweed and sugar beet show a temporal uniform ripening and are therefore „harvested“ at the same time
  - The majority of sugar beets are harvest at a time when the majority of ragweed seeds have already ripened and have fallen from the mother plant

    >> under moist field conditions (October onwards) the huge beet lifters carry – besides the sugar beets – a lot of soil and ragweed seeds with them
  - one counting of BOKU showed a contimation of 7.500 seeds/harvest machine in a medium-contaminated field
  - particularly successfull vectors ragweed seeds are machinery contractors

beet storage
place in
Raasdorf
(Lower Austria)
autumn 2017

Registered herbicides & their efficacy (Bayerische Landesanstalt für LW, 2016)

Compounds for herbicide treatment in wheat, maize, sugar beet, potato and grassland

PRE-EMERGENCE	EARLY POST-EMERGENCE (Ragweed BBCH 12-14)		LATER POST-EMERGENCE (Ragweed BBCH up to 59 before flowering)	
Goltix 700 SC (Metamitron) **Click (Terbuthylazin)** **Arelon Top (Isoproturon)** **Sencor WG (Metribuzin)**	Biathlon (Tritosulfuron) Starane XL (Fluroxypyr) Pointer (Tribenuron) Lontrel (Clopyralid) Callisto (Mesotrione)	**Clio + Dash** (Topramezone) Mais Banvel WG (Dicamba) **Certrol B (Bromoxynil)** Roundup (Glyphosat)	Starane XL (Fluroxypyr) Lontrel (Clopyralid) U46 M-Fluid (MCPA) **U46 D-Fluid (2,4 D)**	Mais Banvel WG (Dicamba) **Certrol B (Bromoxynil)** Simplex (Fluroxypyr) Roundup (Glyphosat)

Results

Click, Arelon Top und Sencor WG prevent the germination of ragweed	Callisto und Clio (4-HPPD-inhibitor) showed a 100% efficacy	limited success of Simplex and Roundup (70-80 %)
Goltix 700 SC reduced ragweed germination only by 35 % compared to the untreated controll	Certrol B first showed a very good effect but promoted new growth a few days after application	even though Starane XL showed quite good results in the early post-emergence, there was only a small effect on the ragweed plants later on
	Sulfonylurea-herbicides like Biathlon und Pointer SX showed no significant effect on ragweed plants	all other herbicides showed no effect

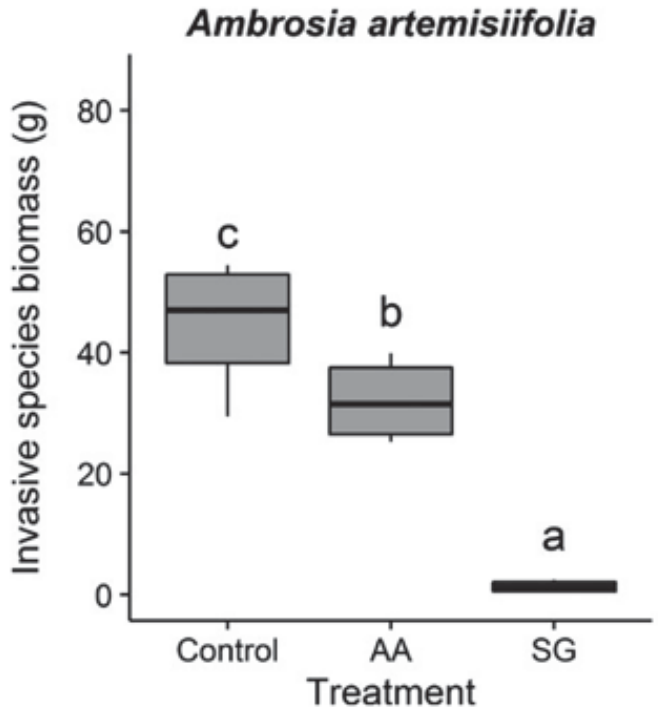
Soybean: only one registered herbicide (Pulsar 40 (Imazamox)) with medium-efficacy

ALTERNATIVE APPROACHES

Competitor seed mixtures

mulit-trait limiting similarity theory

AA	SG
Betonica officinalis Campanula rotundifolia Centaurea scabiosa Dactylis glomerata Galium album Linum perenne Medicago lupulina Peucedanum oreoselinum Teucrium montanum Veronica chamaedrys	Achillea millefolium Agrostis capillaris Buphthalmum salicifolium Campanula rotundifolia Dactylis glomerata Festuca rubra Galium album Poa pratensis Veronica chamaedrys



Yanelli, Karrer & Hall, 2017

Volatile organic coumpounds (VOCs)
Plant-to-Plant-Interaction

- Living plants emit VOCs from organs (leaves, roots etc.) in their surrounding environment but also dead plant residues can release allelopathic substances
- Today, more than 1,700 VOCs have been identified, most of them having the ability to inhibit or stimulate growth of other plants
- Competition between plants has been recognized as one of the most important factors affecting growth of individuals and the distribution of species (chemical behaviour)
- **Enemy release hypothesis** states that invasiveness of non-native species like ragweed can result from a loss of natural enemies due to the production of toxic compounds (i.e. growth inhibitors) that unfavorably affect native communities >> native plants are unable to tolerate compounds released by a non-native plant due to missing co-evolution in the same environment
- Ragweed: 53 compounds, 10-15 active; toxic substances like neurotoxines, as well as positive substances like substances having the potential to cure haemorrhoides

Communication is no one-way-street

Ophrealla communa

- 3-4 mm long
- Caput is yellow with a black spot on the back
- fluffy
- temperature optimum: 25-28°C
- native to North America too
- up to 6 generations per year
- **favorite food:** ragweed >> all life stages (eggs, larvae, adults) live on the plant and have therefore the potential to harm it severely
- **BUT:** What happens when the beetle has eradicated all ragweed?
- **Next possible snack bars:** sunflower and other Asteraceae (nature conservation issues???)

How to manage the Ambrosia plant?

1) Depletion of the soil seed bank

2) Prevention of seed formation and avoidance of further spread of existing seeds

- Bare fallow with shallow soil management to „motivate“ seeds to germinate
  proper monitoring and management of this stands are essential
  >> very useful for smaller sites with low-medium seed contamination as approximately 85 % of the seeds germinate the year after ripening

- targeted soil management against ragweed (hoeing, grooming etc.) is possible until BBCH 18 (crops) only >> after that: uprooting if possible

- Mulch sowing (weed suppressing effect)

- as viability slowly but steady decreases, farmers should focus on competitive autumn-sown crops (wheat, rape seed etc); particularly on heavy contaminated fields spring-sown crops like soybean or sunflower with low competitive power should be avoided

How to manage the Ambrosia plant?

- targeted actions against ragweed not only along roadside verges and agricultural fields but also on low-interest-sites (ruderal areas)
- **Attention:** high regenerative capacity >> a regular monitoring of the areas after the management event is essential >> in most cases repetitive actions are necessary since „under pressure“ the plant can build up new seeds within 5 weeks
- Dirty machinery (soil/biomass etc) should be cleaned each time before the enter a new field/road/ruderal area
- Education and communication

But all previouse experience show:

Once ragweed = always ragweed

And there is still no end in sight....

Ambrosia psilostachya and the hybrid (*A. artemisiifolia x psilostachya*)

Thank you for your attention!

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My notes

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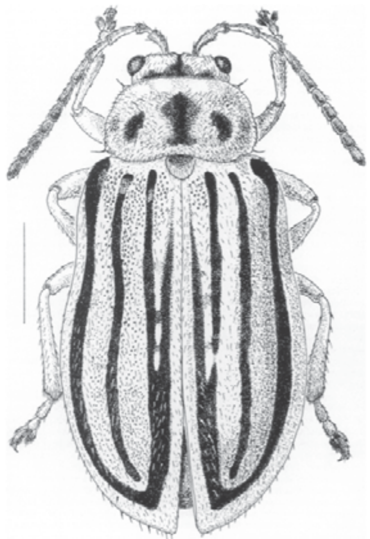
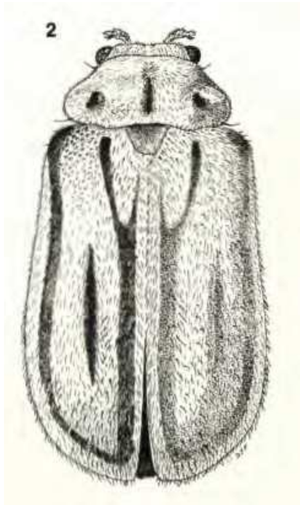
(1) Morphology and Biology

Ophraella Slobodkini, female	Ophraella communa, female
 (Futuyma, 1991)	 (Futuyma, 1990)
4 dark stripes on the elytra = vittae (from the midline on: subsutural, supplementary, discal, lateral)	4 dark stripes on the elytra = vittae (from the midline on: subsutural, supplementary, discal, lateral)
Supplementary vitta joins the subsutural more than halfway toward apex	Supplementary vitta joins the subsutural less than a third toward the apex
Discal vitta originates near the base and terminates near the apex	Discal vitta originates farther from the base and terminates farther from the apex

Futuyma D.J. 1991. A New Species of Ophraella Wilcox (Coleoptera: Chrysomelidae) from the Southeastern United States. Journal of the New York Entomological Society, Vol. 99, No. 4, pp. 643- 653

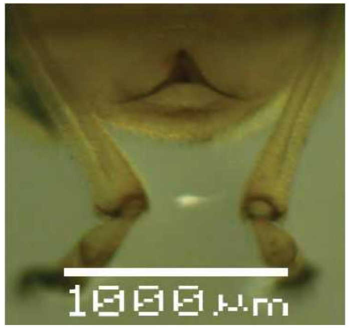
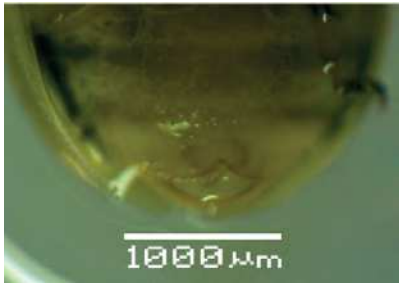
Futuyma D.J. 1990. Observations on the taxonomy and natural history of Ophraella wilcox (Coleoptera: Chrysomelidae), with a description of a new species. Journal of the New York Entomological Society, Vol. 98, No.2, pp. 163-186

Species traits

Body length of adults
3.4–4.1 mm males
3.9–4.3 mm females.
head is yellowish, with dark brown spots at the back.
Body is coarsely punctured. Antennae are dark brown. Pronotum is yellowish or pale brown, with three black or dark brown spots.
Elytra are yellowish or pale brown and show dark brown longitudinal stripes
Eggs are laid on the underside of young leaves of the host plants; first yellow, later orange.
Before the pupation, the beetles form cocoons on a leaf tip. Pupation lasts one to two weeks.

Differences between males & females

Bachelor thesis FS 2017 — Liu Shing-Chi

Ophraella communa male	Ophraella communa female
	
„V"- stripe at the end of genital abdominal segment (Guo, 2013)	No „V"-Stripe at the end of genital abdominal segment (Guo, 2013)
male front 	female front 
male back 	female back 
Number of antenna segment: 11	Number of antenna segment: 11
Length of antenna: 2.5 ± 0.16 mm	Length of antenna: 2.7 ± 0.07mm
Body height: 4.19 ± 0.22 mm	Body height: 4.9 ±0.43 mm
Body width: 2.03 ± 0.14 mm	Body width: 2.4 ± 0.14 mm

Guo W., Zhou Z, Gua JY, MA J. 2010. Morphological characteristic of adult Ophraella communa LeSage. Plant Protection 5, 179-182.

Figure 1. A female O. communa, B male O. communa (pictures from Janisse Deluigi)

To prepare the old and young adults for the experiment, pupae are collected and isolated in a petri dish. When the new adults emerged, they were sexed, and isolated in a 15ml Falcon® tube (Figure 2) with a leaf of *Ambrosia artemisiifolia* for the supply and dated. In total 86 old males and 110 young females have been isolated for the experiment (Figure 2).

Figure 2 Falcon's tubes for containing O. communa

(2) Life stages

L1

L1-L2

L2

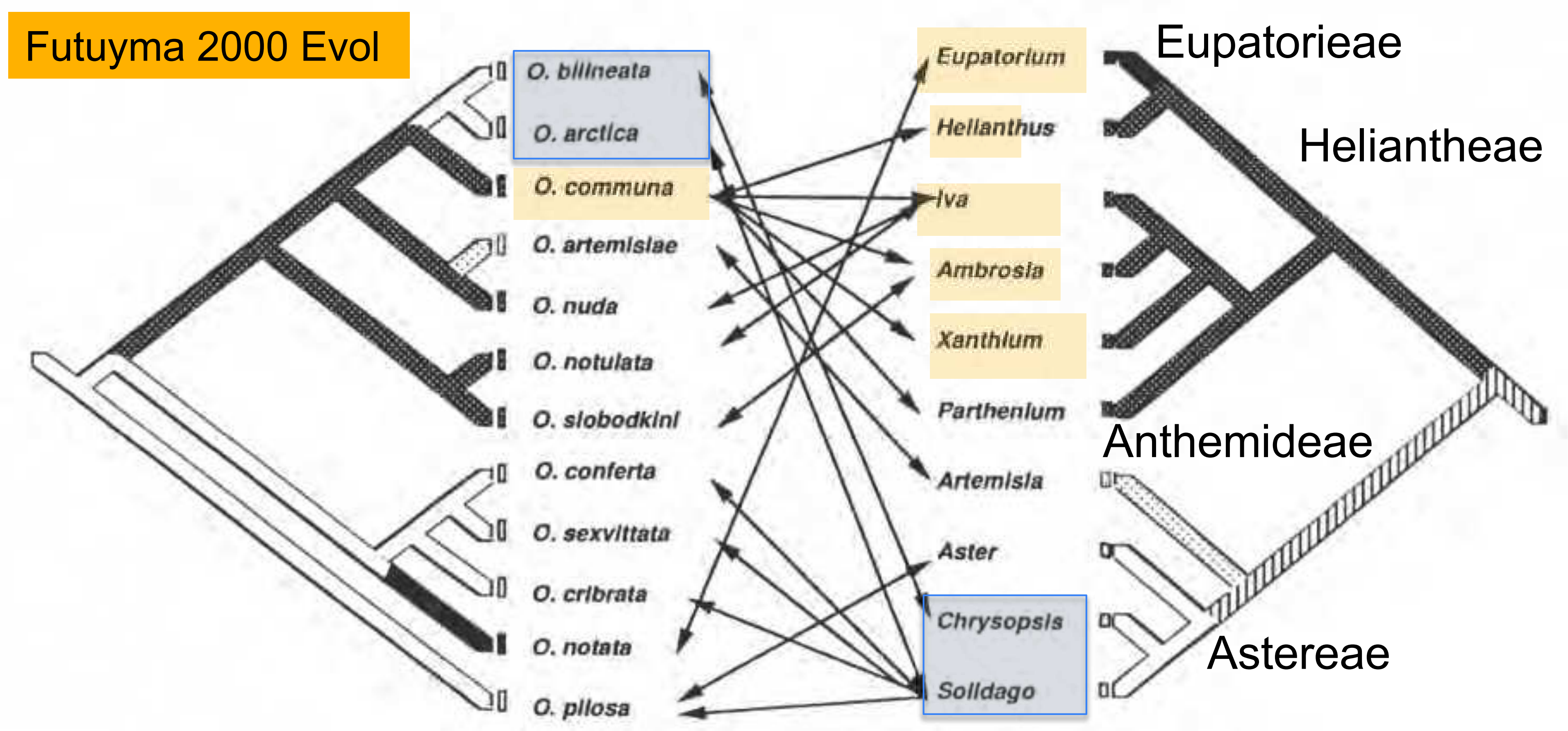
L3

Pupa

Age: 1 day, 4days

(3) Phylogeny

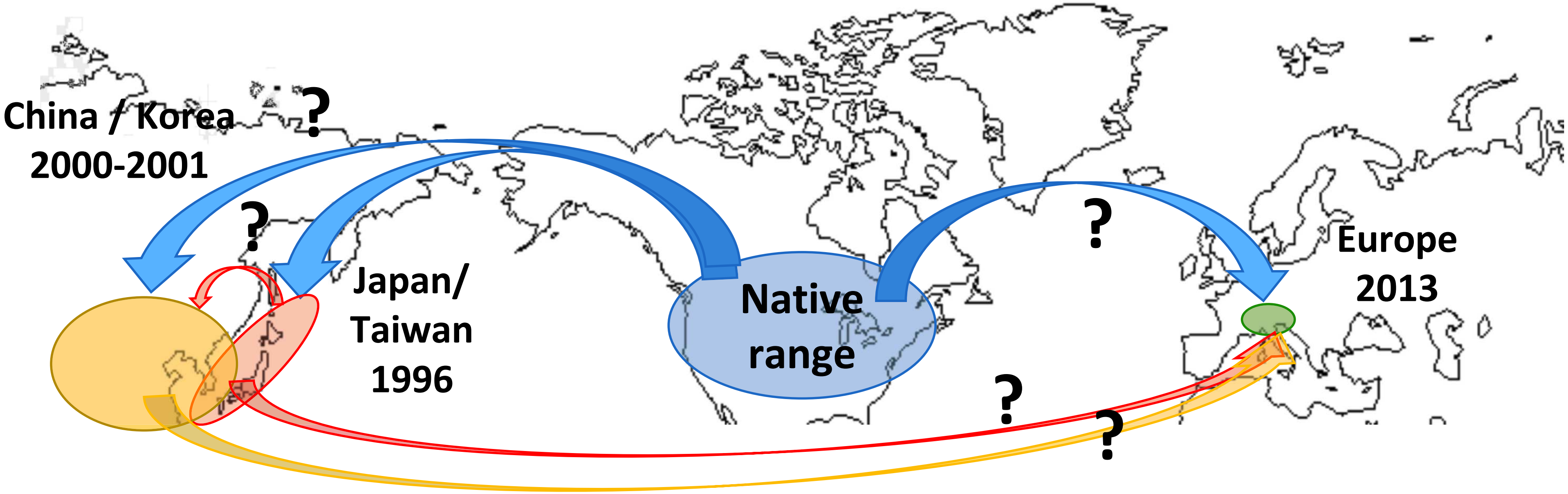
Ophraella (N-American genus) with14 described species
all are host-specific feeders on plants in 4 tribes of Asteraceae



➜ Plants speciated long before the beetles (incongruent phylogeny)

(4) Invasion history, Ecology and Distribution

Ophraella communa: invasion history



The species is oligophagous, but both adults and larvae feed preferentially on *Ambrosia artemisiifolia*

68 populations selected

5 ind./pop

Beetles sampled on :

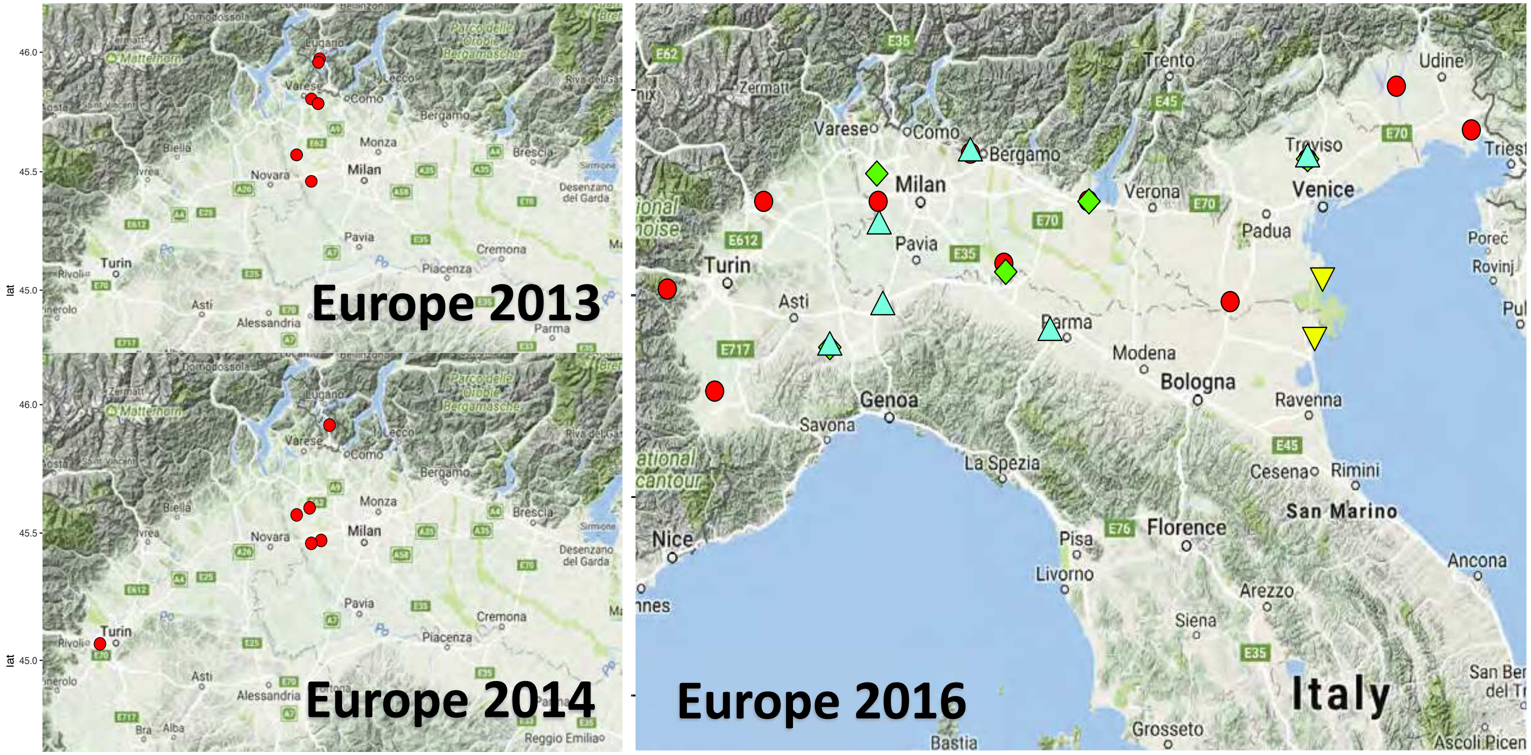
A. artemisiifolia

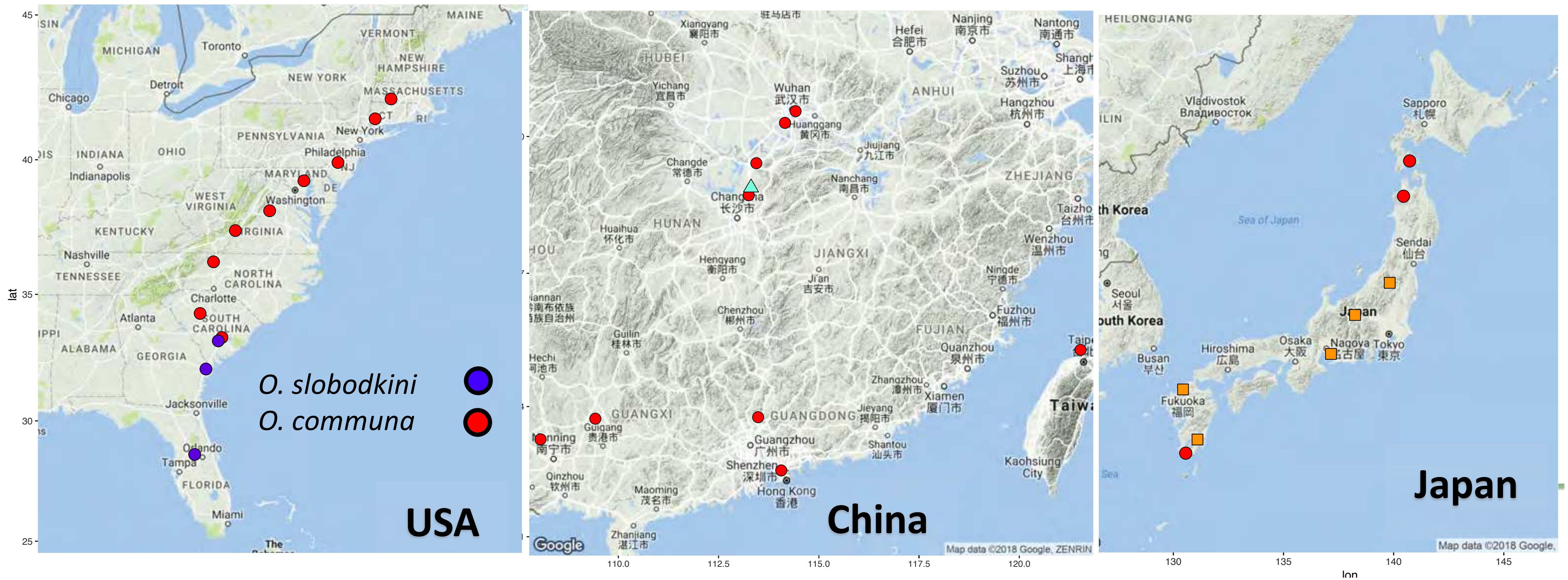
A. trifida

A. psilostachya

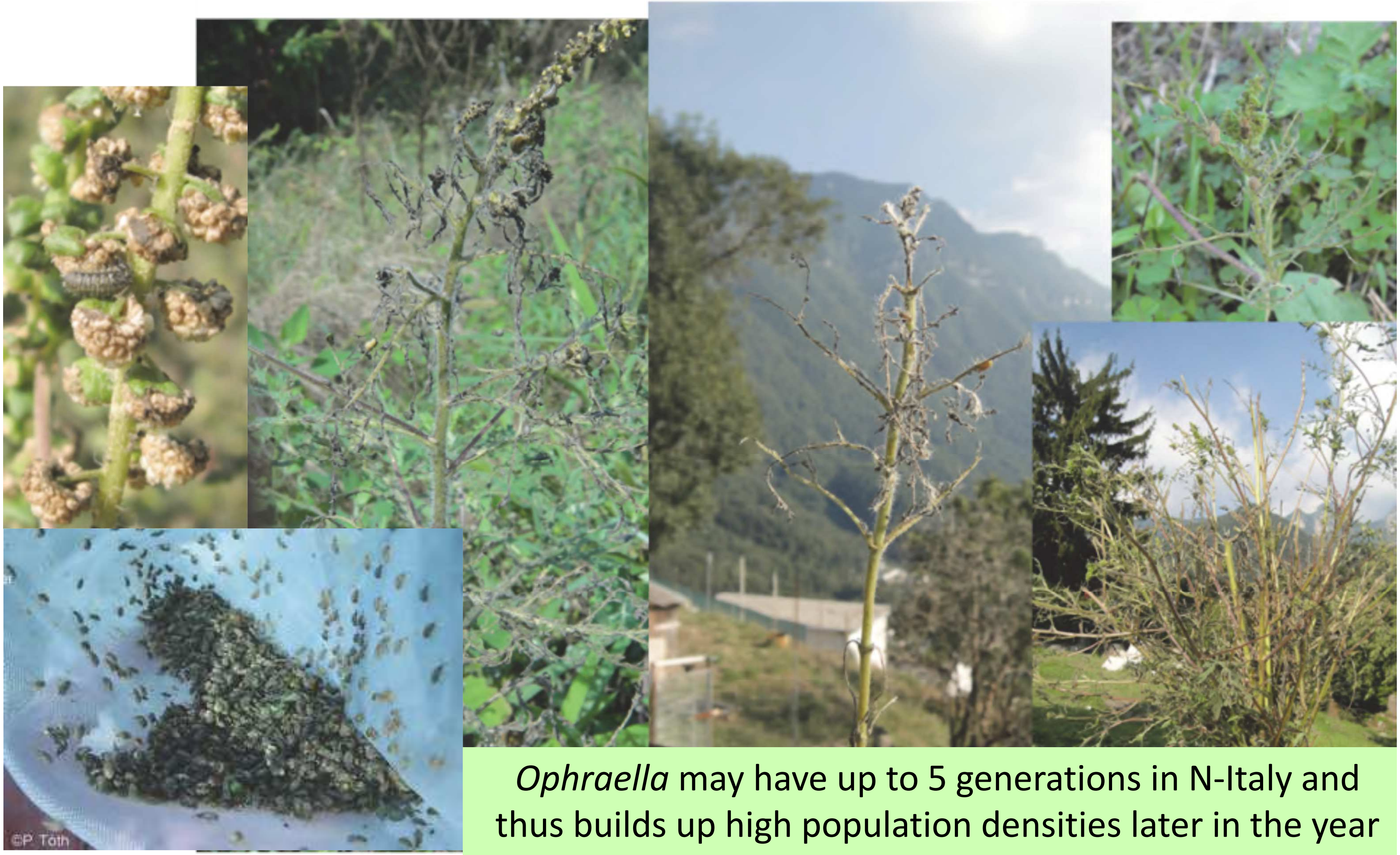
H. tuberosus

Xanthium sp.



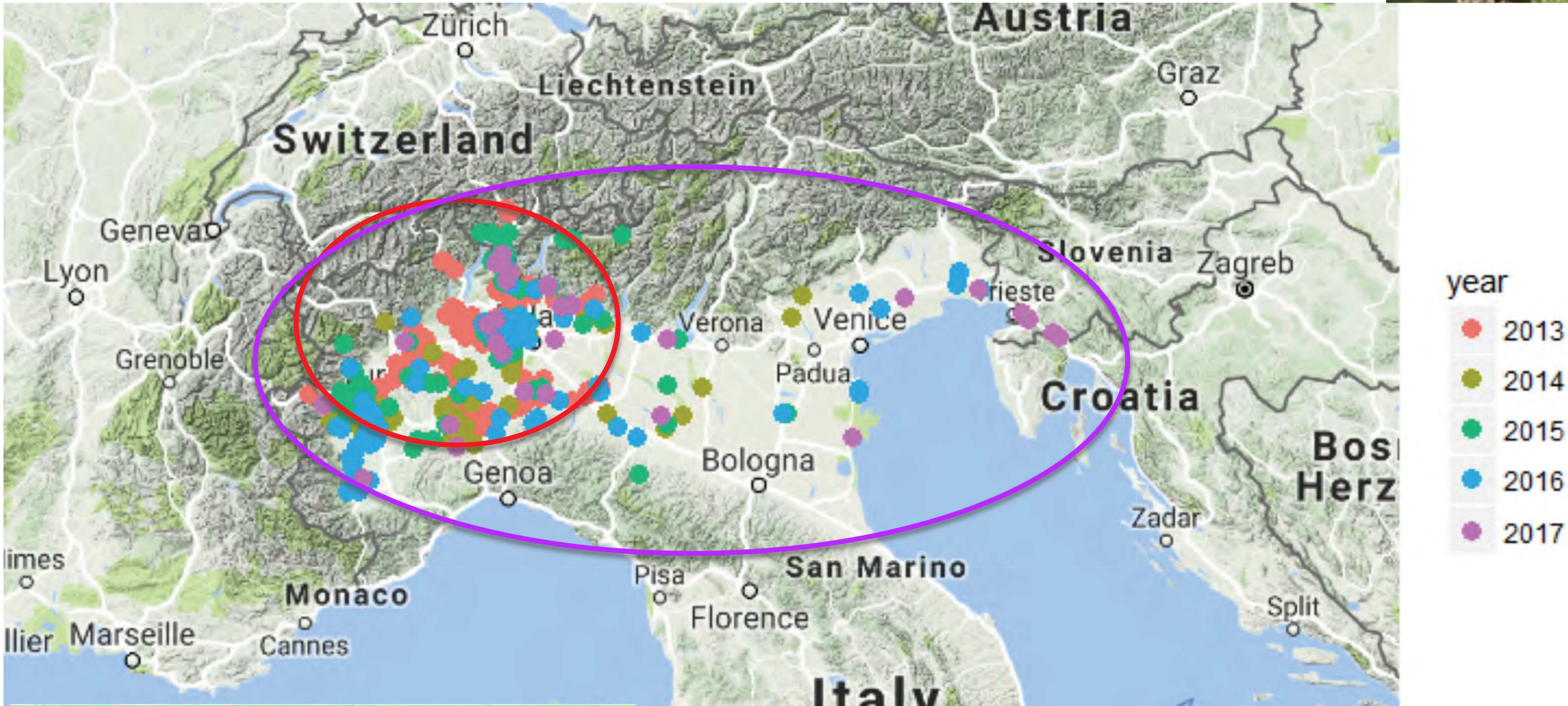


Damage by *Ophraella communa* on ragweed can be high



Ophraella may have up to 5 generations in N-Italy and thus builds up high population densities later in the year

Fast spread from 2013 to 2017



WEED RESEARCH

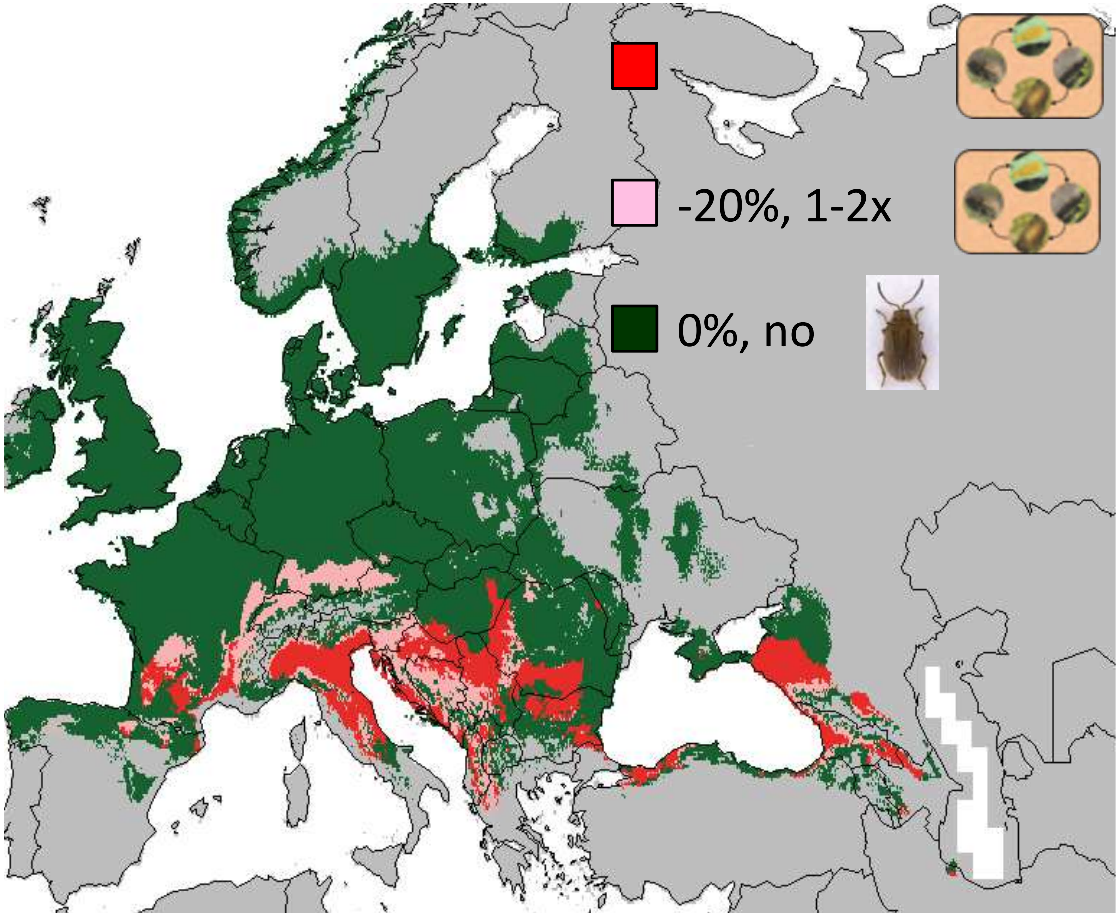
PRIORITY PAPER

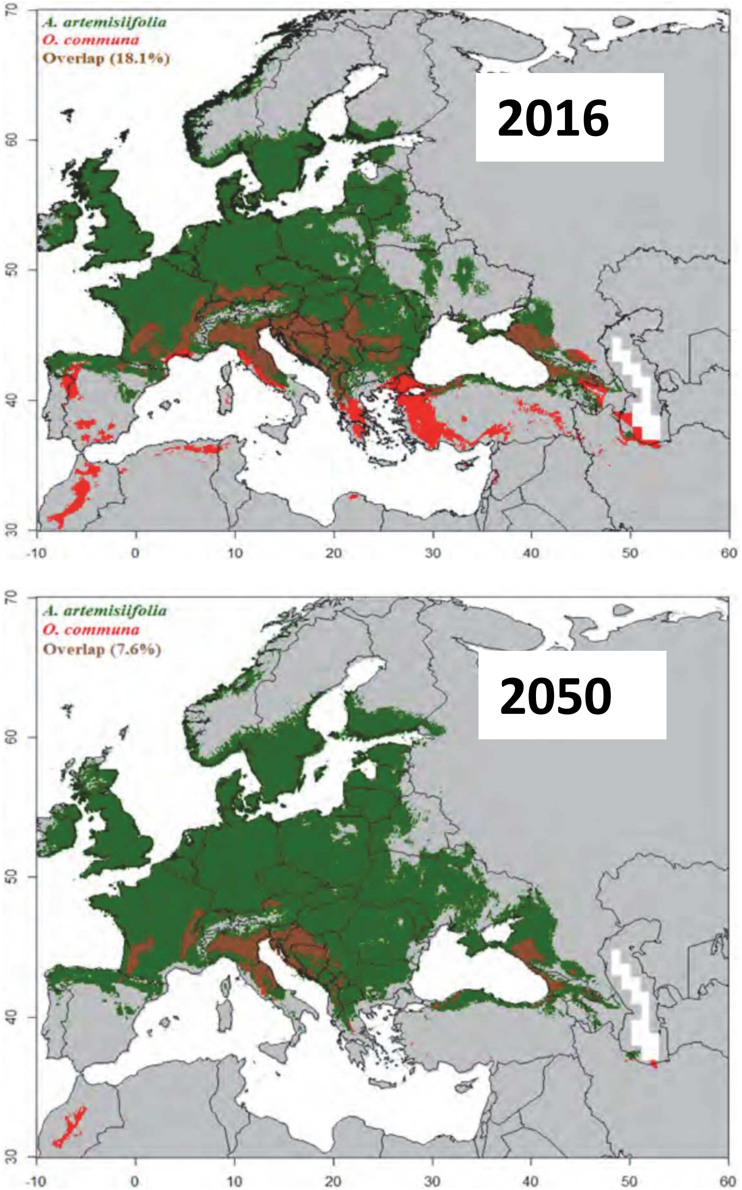
***Ophraella communa*, the ragweed leaf beetle, has successfully landed in Europe: fortunate coincidence or threat?**

H MÜLLER-SCHÄRER*, S T E LOMMEN*, M ROSSINELLI†, M BONINI‡, M BORIANI§, G BOSIO¶ & U SCHAFFNER**

In September 2017, we found *Ophraella* for the first time in Slovenia and Croatia, but it has not yet been recorded from France and Austria

Predicted number of generations and cover of common ragweed infestations today and under climate change





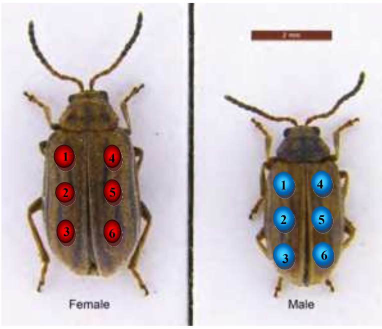
Sun et al. 2017; Ecosphere

(5) Experimental set-up to study host specificity and impact

Immobilizing *Ophraella* to allow marking and measuring traits

Glass Petri dish + ice + filter paper

Marking individuals using nail polish



Combination	*Day*	*F*	*M*	*Combination*	*Day*	*F*	*M*
1				21 = {3 ,6}			
2				22 = {1, 2, 3}			
3				23 = {1, 2, 4}			
4				24 = {1 ,2 ,5}			
5				25 = {1 ,2 ,6}			
6				27 = {1, 3,4}			
7= {1 ,2}				28 = {1 ,3 ,5}			
8 = {1 ,3}				29 = {1 ,3 ,6}			
9 = {2 ,3}				30 = {2, 3,4}			
10 = {4 ,5}				31 = {2, 3,5}			
11 = {4 ,6}				32 = {2, 3,6}			
12 = {5 ,6}				33 = {2, 4,5}			
13 = {1 ,4}				34 = {2, 4,6}			
14 = {1 ,5}				35 = {3, 4,5}			
15 = {1 ,6}				36 = {3, 5,6}			
16 = {2 ,4}				33 = {4, 5,1}			
17 = {2 ,5}							
18 = {2 ,6}							
19 = {3 ,4}							
20 = {3 ,5}							

Tests under controlled conditions (in our quarantine facility)

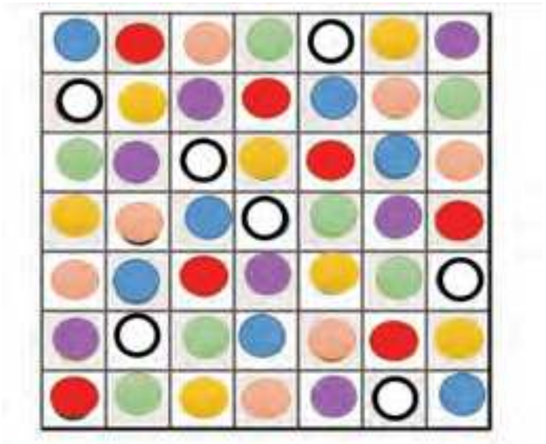
- 3-7 plant species with 2-4 ind. / cage
- in the presence and absence of ragweed
- Transfer of
  - adults ➔ oviposition **preference**
  - eggs/larvae ➔ **performance**

Cat boxes

In total some 50 tests 2014 – 2017
- 6 ragweed species
- 4 ornamentals
- 4 sunflower varieties,
- 9 native endangered species

Clip-on cages for single larvae

Tests in the field (at our study sites in Magnago near Milan)



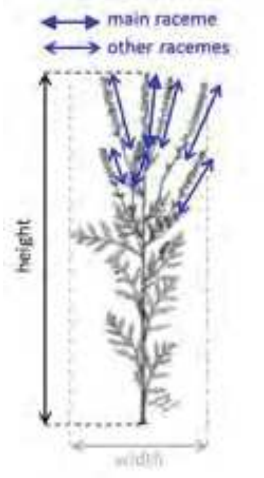
Latin square design

- over two years
- at 4 sites
- in 3 cohorts (early May, mid-July & early Sept.)
- 5 assessments/cohort
- various sunflower cultivars (for oil, as ornamental or green manure)

Establishing latin square design on 1 May 2015 in Italy

Studying impact at the population level by using insecticides to exclude *Ophraella*

Biomass
N=20



Raceme length
N=20

Flower heads
N=10

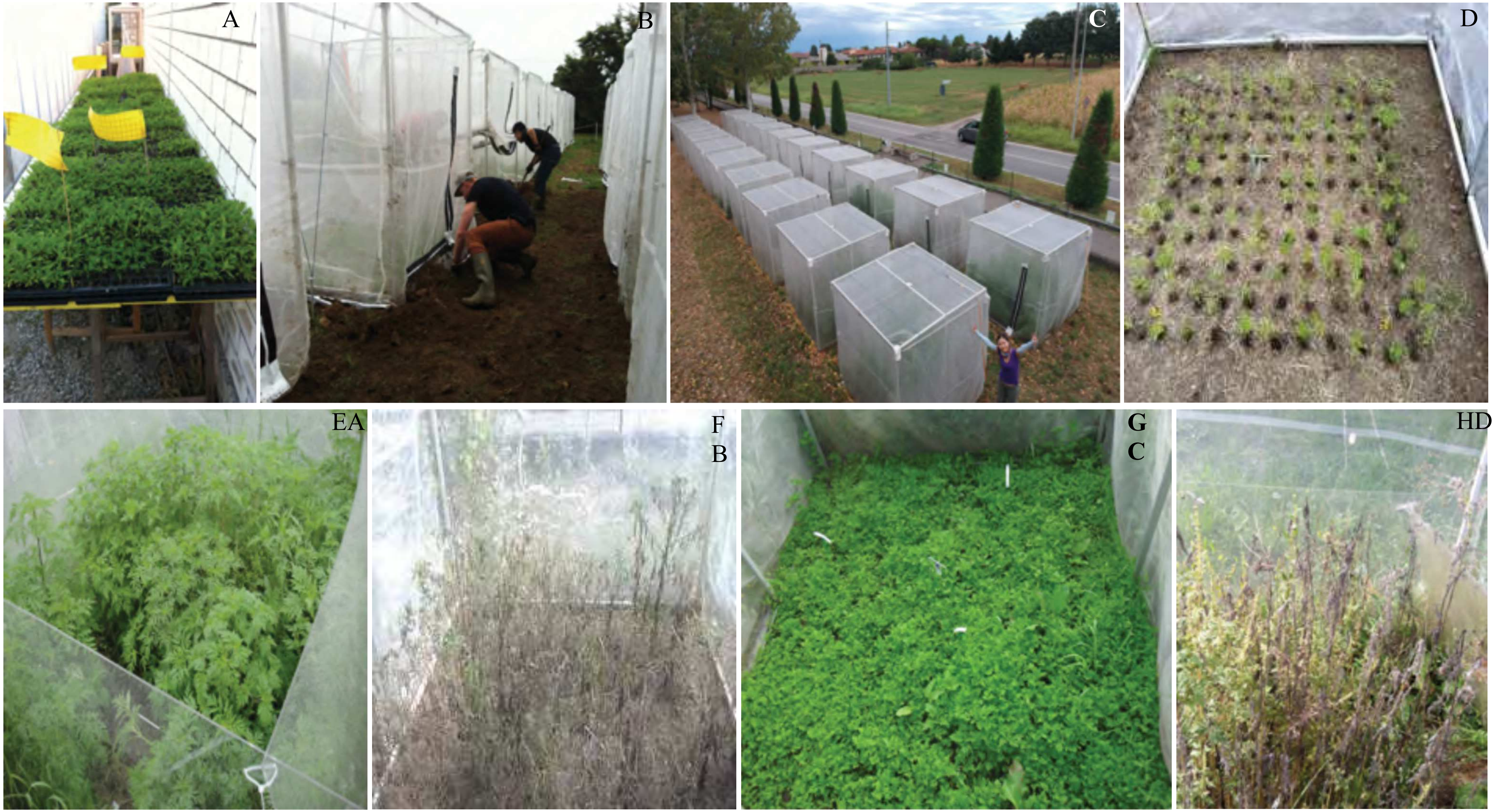
Flowers
N=10

Pollen

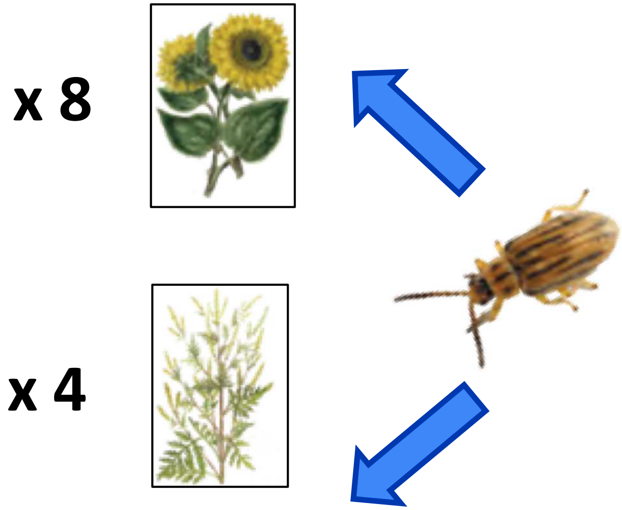
- Quantity
- Quality

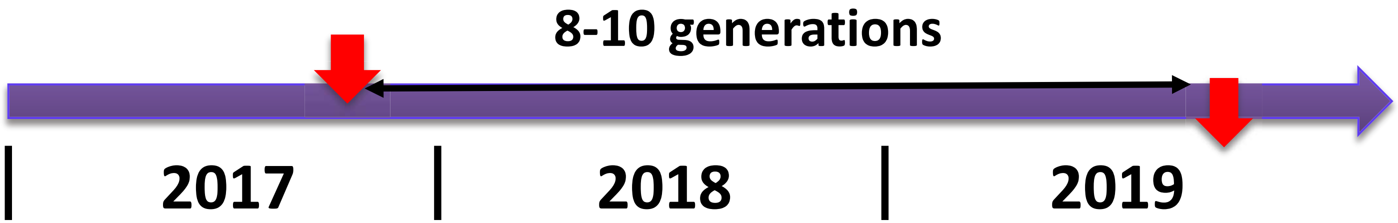
Experimentl evolution studies:

(i) impact of *Ophraella* on artificial ragweed populations under climate warming assessed by genomics and phenotyping



(ii) assessing differences at the phenotypic and genomic levels after 10 generations of evolution on sunflower





Phenotypic analyses

Bioassay in the Qlab
Adult host choice and larval performance

+

Pop genomic analyses

Poolsequencing
Investigate the variants involved in adaptation

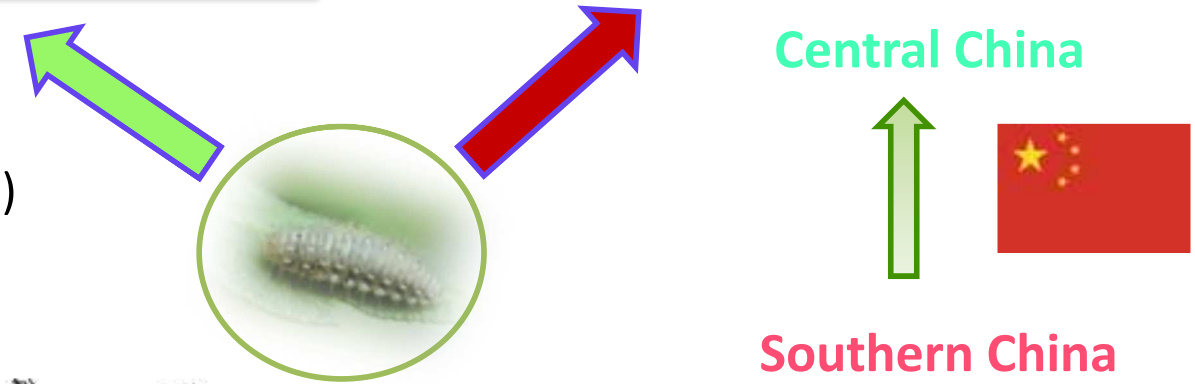
Phenotyping to explore phenotype-environmental correlations

Do *O. communa* populations differ in their performance on different **host plants**?

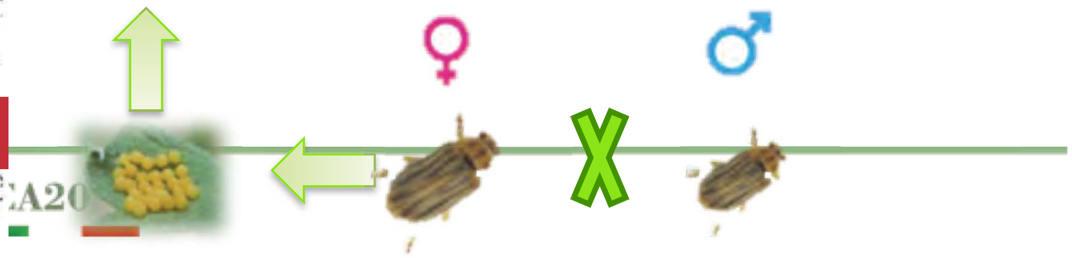
… differ in their performance when exposed to different **temperatures**?

Ambrosia artemisiifolia | *Helianthus annuus* (P64H42 variety) | *Ambrosia trifida*

20°C | 26°C | 31°C



- Native and introduced ranges
- 12 populations (4 per range)
- Different longitudinal gradients



Plant invasions and biological control

offer a great natural laboratory for studying

(i) ecological and evolutionary processes, and

(ii) outcomes of species interactions under changing environments

This is relevant for predicting

(i) Potential benefits and costs of biological control measures

(ii) future spread of species under climate change, and

(iii) for making invasive species management more predictive

Thank you for your interest

International Ragweed Society

"Look deep into nature, and then you will understand everything better"

Albert Einstein

My notes

My notes

My notes