New strategies for monitoring pests in (sub-)tropical crops: Insights from **DNA Barcoding** and Field Surveys

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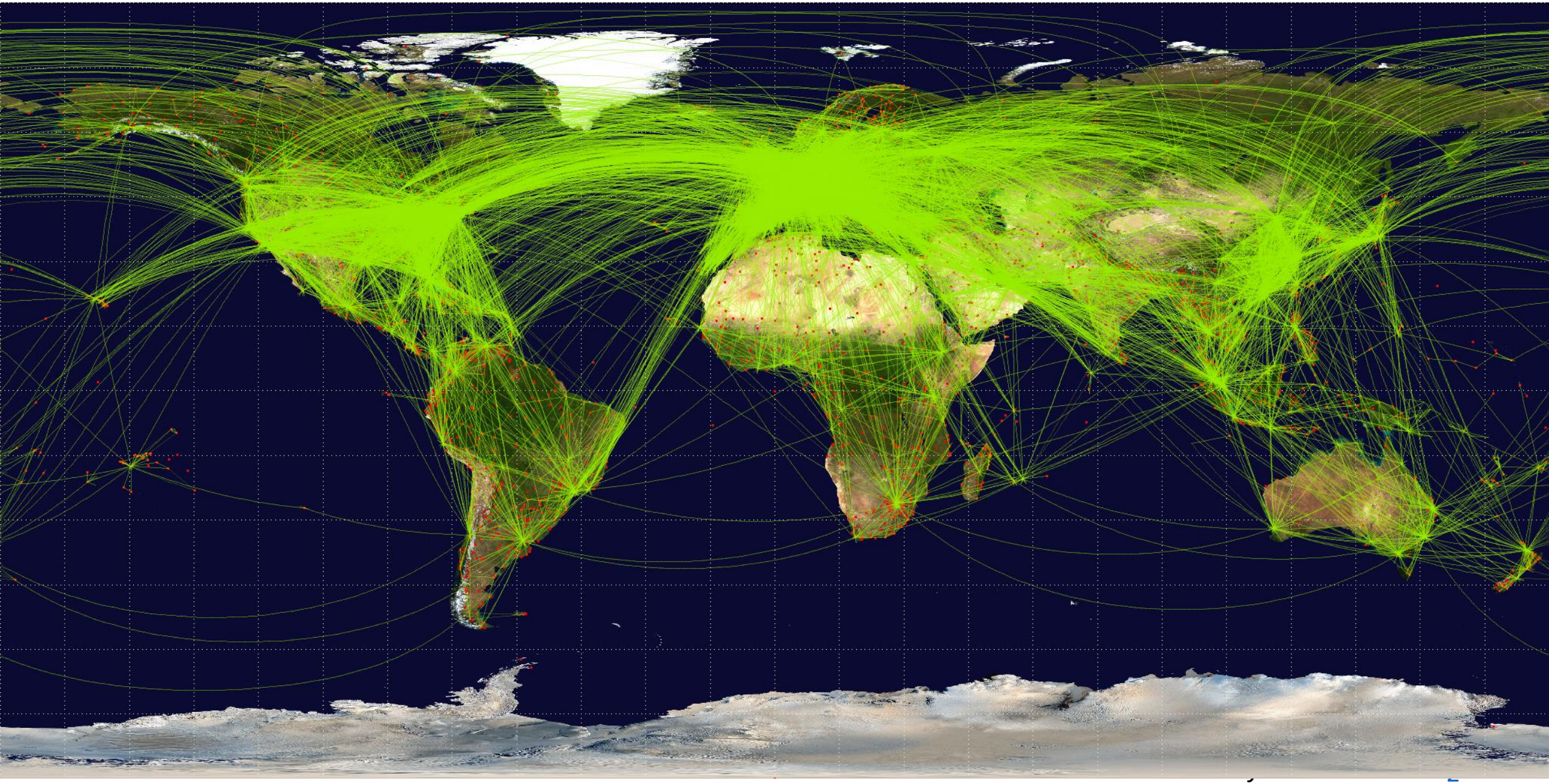
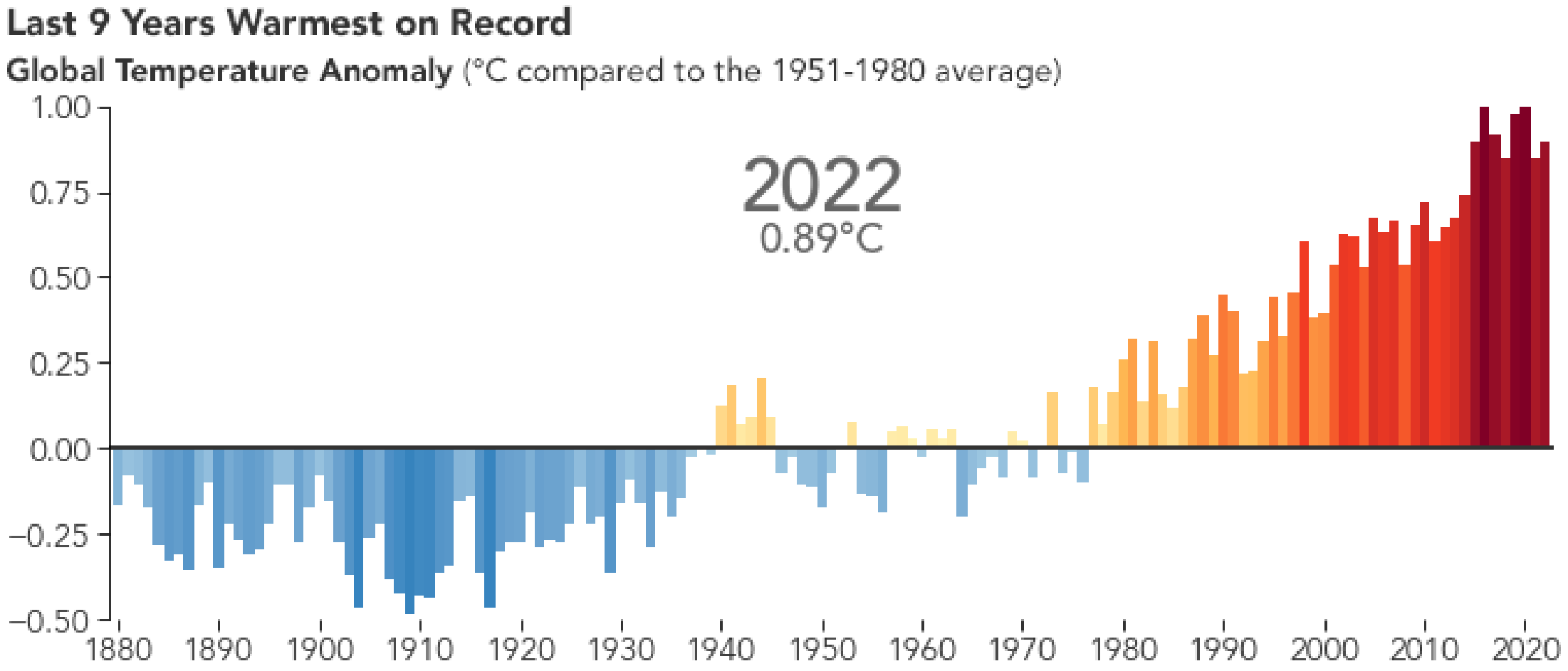
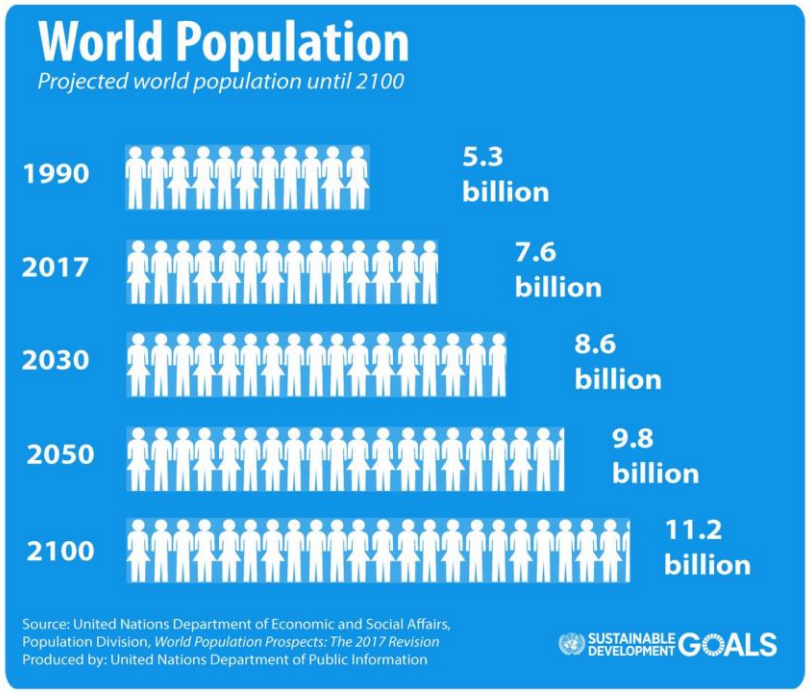


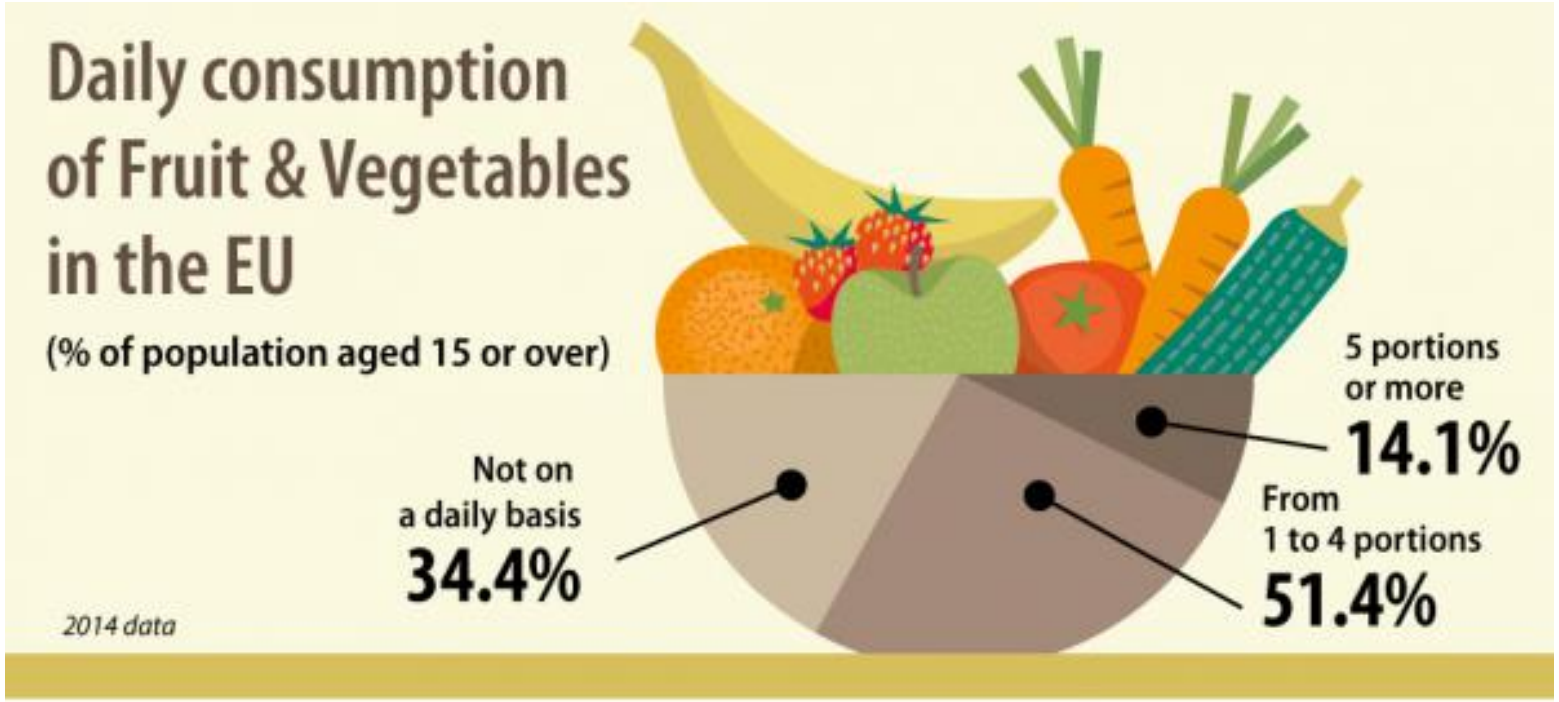
Climate change



Data source: NASA

Global food systems

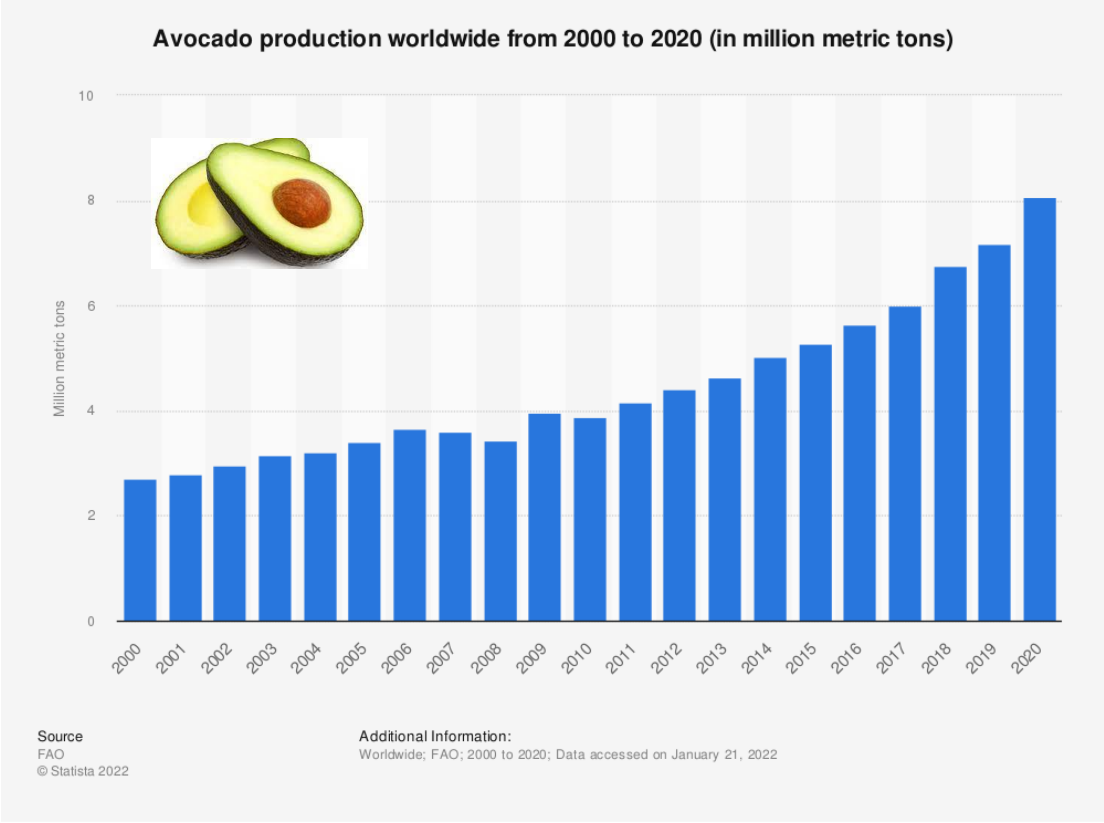




Commodities

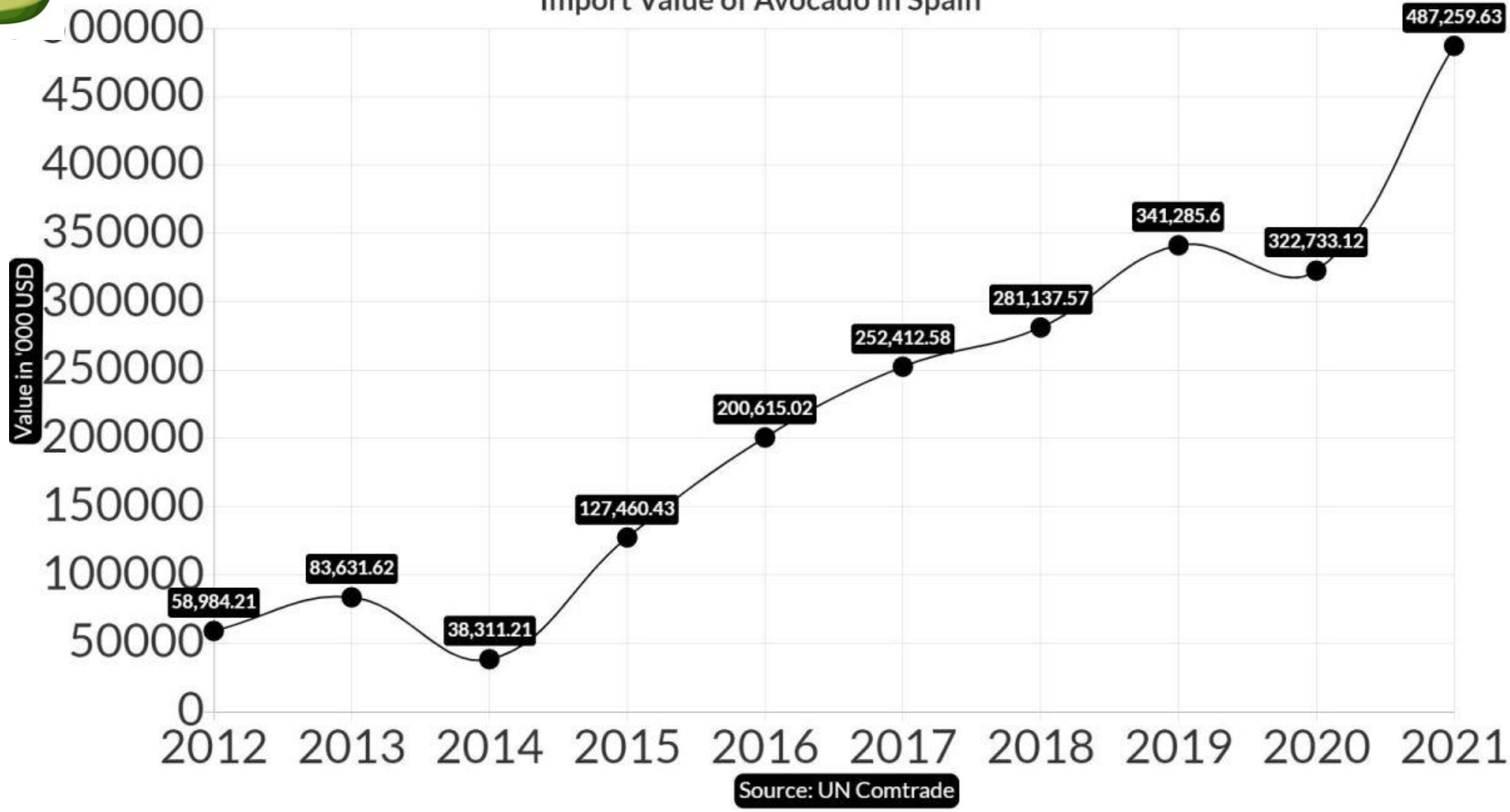
The import value of exotic fruits in Europe (e.g., pineapple, avocado, mango, lychees, passion fruits, carambola and pitahaya) increased 40% over the past five years. Export volumes of the three major fresh tropical fruits – mango, avocado and papaya have displayed the fastest average annual growth rates among internationally traded food commodities in recent years. Avocado is expected
to become the second-most traded major tropical fruit by 2030, after bananas. (FAO 2022)

Increasing demand



Data source: FAO

Import Value of Avocado in Spain



Pressure on production areas

Drastic land -use changes last 10 years

Consequences

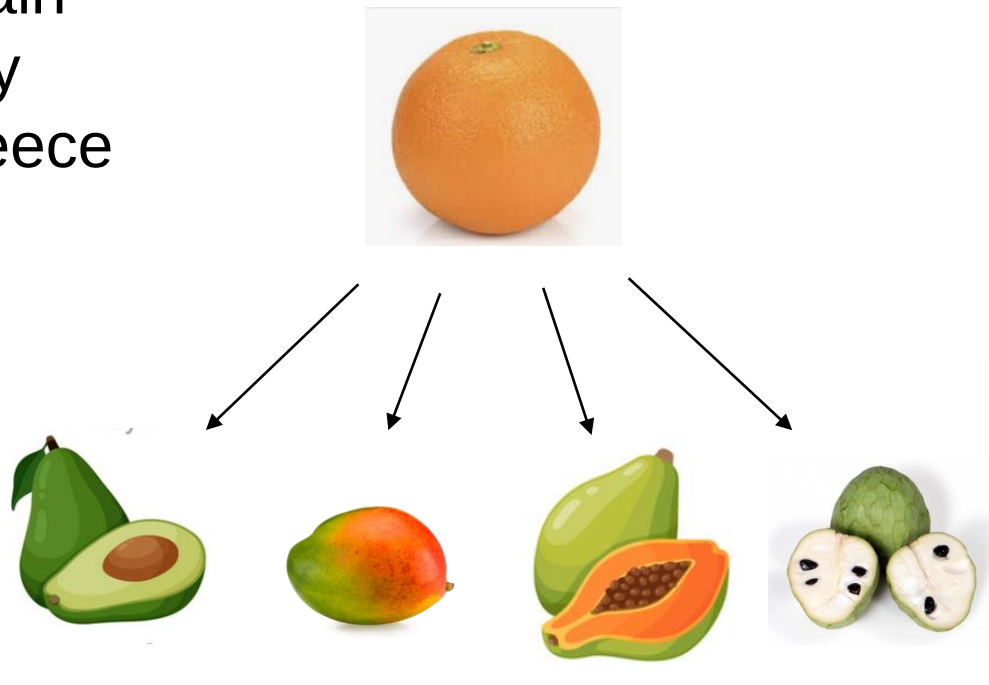
Import of (fresh) fruit

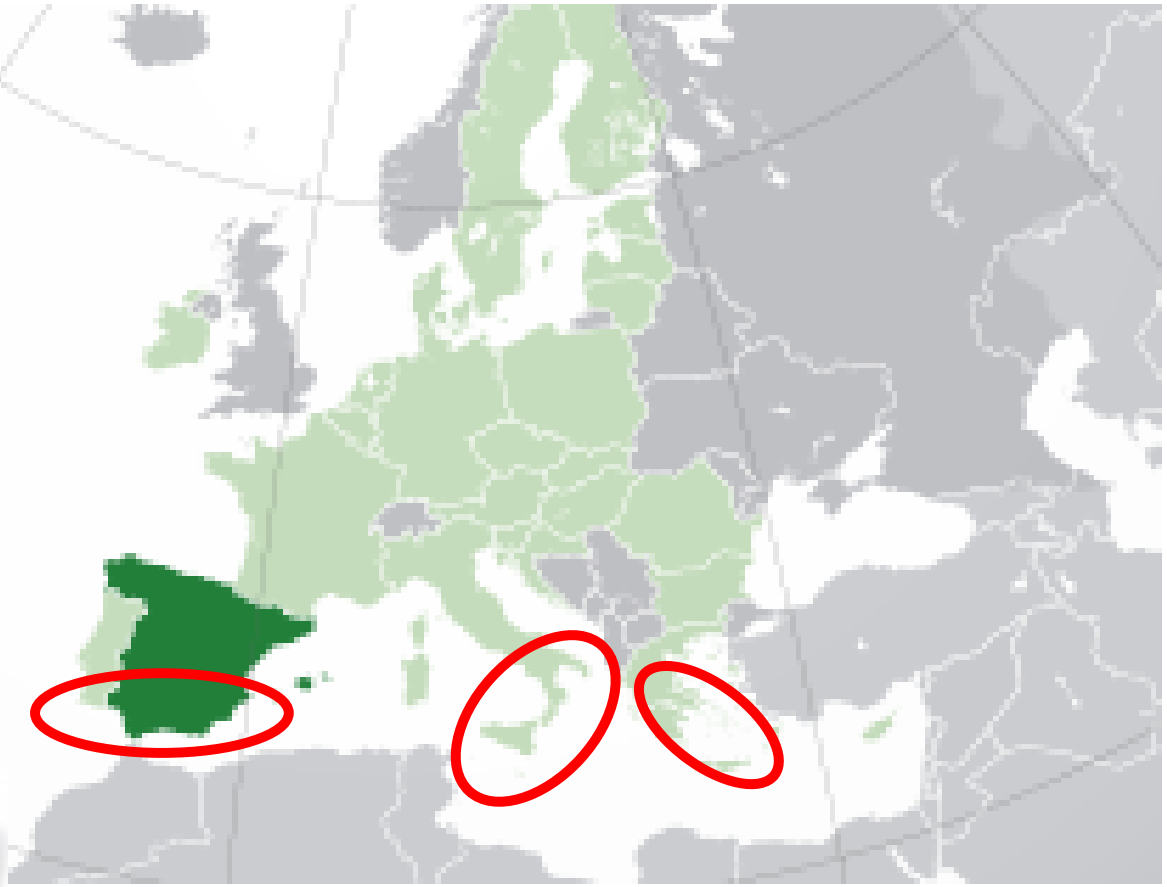
Introduction of propagation material

Subtropical crops

Production of subtropical crops spreading and diversifying:

- Portugal
- Spain
- Italy
- Greece






Producción de aguacate en España
Plantae
La Coruña
Lugo
Asturias
Santander
País Vasco
Pontevedra
Tarragona
Castellón
Valencia
Islas
Baleares
Alicante
Murcia
Huelva
Sevilla
Almería
Cádiz
Granada
Málaga
Islas Canarias
Alta
Media
Baja
Nula

Subtropical crops

Regulation

Commission Implementing Regulation (EU) 2018/2019 of 18 December 2018 establishing a provisional list of high risk plants, plant products or other objects, within the meaning of Article 42 of Regulation (EU) 2016/2031

Plants for planting, other than seeds, *in vitro* material and naturally or artificially dwarfed woody plants for planting,of *Acacia* Mill., *Acer* L., *Albizia* Durazz., *Alnus* Mill., *Annona* L., *Bauhinia* L., *Berberis* L., *Betula* L., *Caesalpinia* L., *Cassia* L., *Castanea* Mill., *Cornus* L., *Corylus* L., *Crataegus* L., *Diospyros* L., *Fagus* L., *Ficus carica* L., *Fraxinus* L.,Hamamelis., *Jasminum* L., *Juglans* L., *Ligustrum* L., *Lonicera* L., *Malus* Mill., *Nerium* L. , *Persea* Mill., *Populus* L., *Prunus* L., *Quercus* L., *Robinia* L., *Salix* L., *Sorbus* L., *Taxus* L., *Tilia* L., *Ulmus* L., and plants of *Ullucus tuberosus* Loz., are known to host commonly hosted pests known to have a major impact on plant species which are of major economic, social or environmental importance to the Union. Those plants are also known to commonly harbour pests without showing signs of infection, or to have a latent period for the expression of those signs. T

High Risk Plants

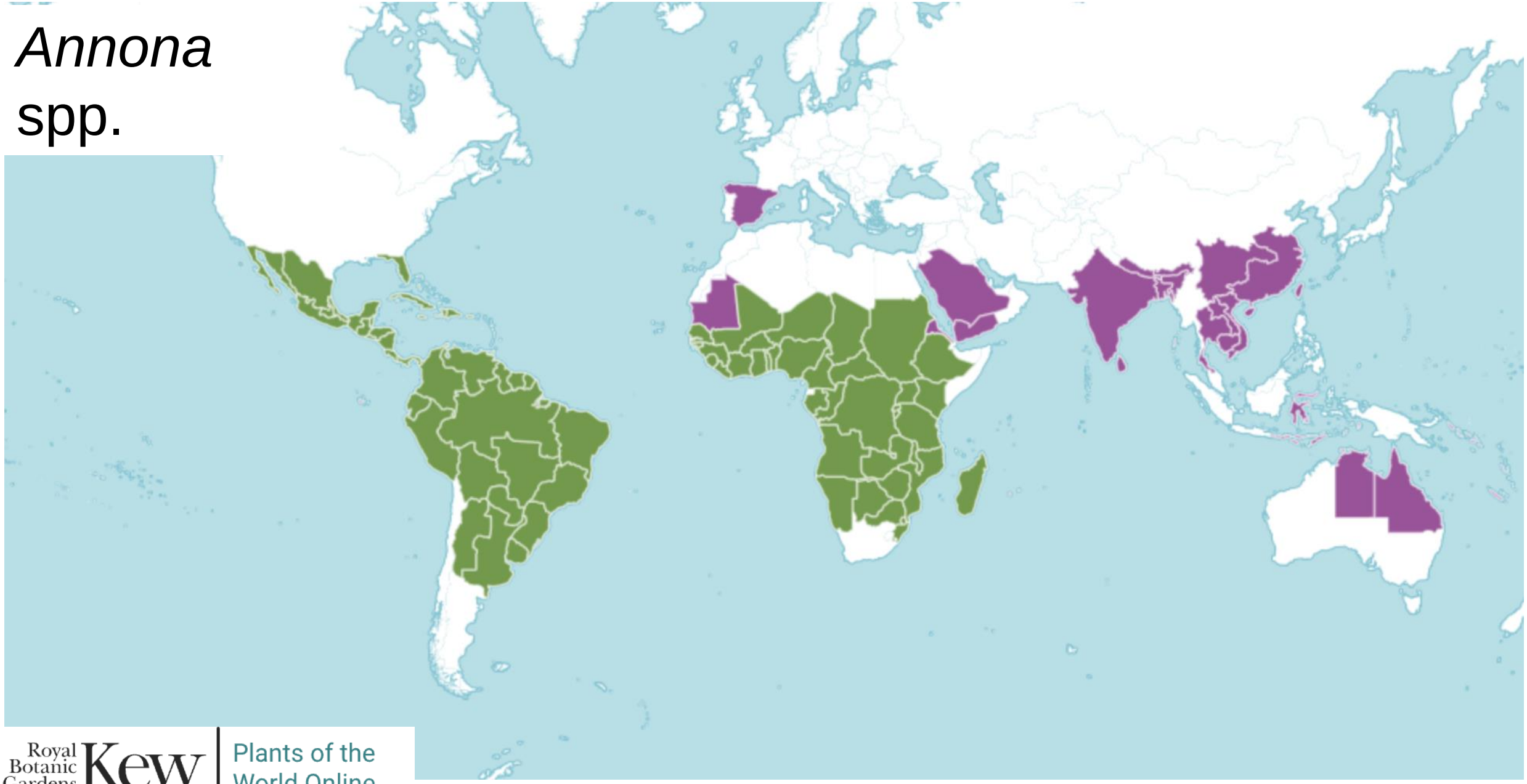
European Commission

efsa

Pest	Family	Regulated
Pulvinaria psiddi	Coccidae	Proposed as q-pest
Protopulvinaria pyriformis	Coccidae	No
Ceroplastes floridensis, C. sinensis	Coccidae	No
Parasaissetia nigra	Coccidae	No
Milviscutulus mangiferae	Pseudococcidae	Proposed as q-pest
Nipaecoccus viridis	Pseudococcidae	Proposed as q-pest
Pseudococcus longispinus, P. cryptus[+]	Pseudococcidae	[+]Proposed q-pest
Planococcus citri	Pseudococcidae	No
Maconellicoccus hirsutus	Pseudococcidae	Proposed as q-pest
Paracoccus marginatus	Pseudococcidae	Proposed as q-pest
Icerya seychellarum, I. aegyptiaca, I. purchasi	Monophlebidae	Proposed as q-pests
Aulacaspis tubercularis	Diaspididae	Proposed as q-pest
Aonidiella orientalis	Diaspididae	Proposed as q-pest
Scirtothrips dorsalis	Thripidae	Q-pest; Priority pest
Retithrips syriacus	Thripidae	Proposed as q-pest
Euwallacea fornicatus	Curculionidae	Q-pest

Annona spp. (custard apples, sweet apples, soursoup)

Annona spp.



Royal Botanic Gardens Kew | Plants of the World Online

Pests

Mango pests

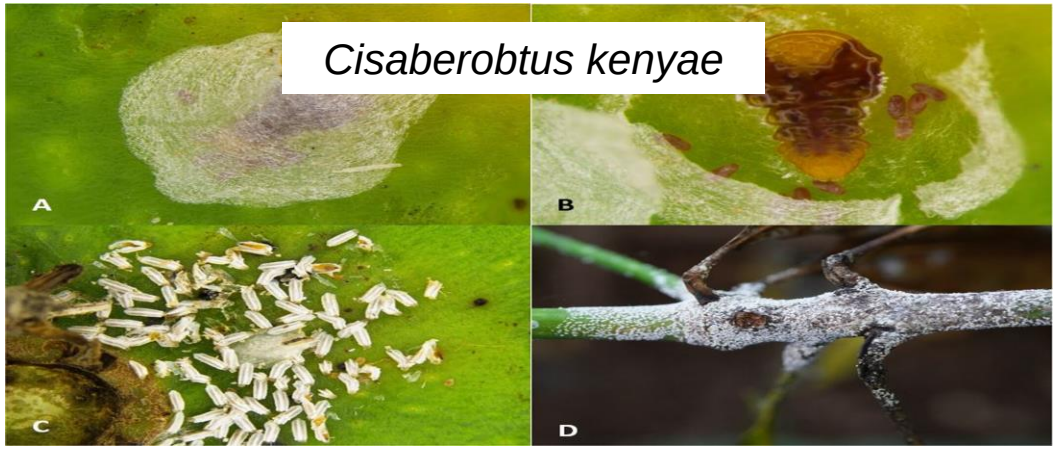
Sternochetus mangiferae

Aulacaspis tubercularis

Pulvinaria psiddi

Icerya seychellarum

Cisaberobtus kenyae



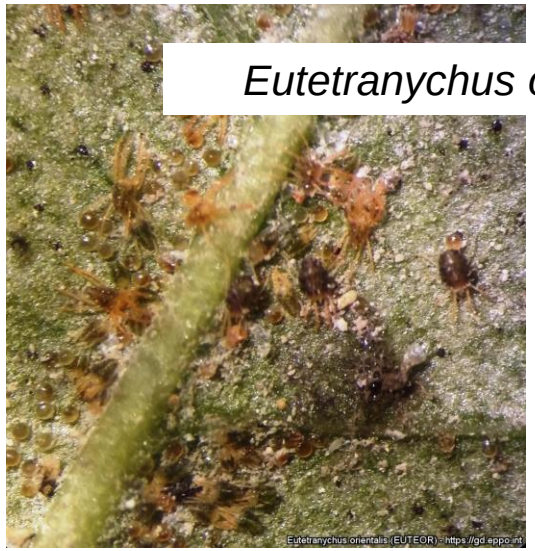
Pictures: EPPO & IVIA

Mango pests: *Scirtothrips dorsalis*

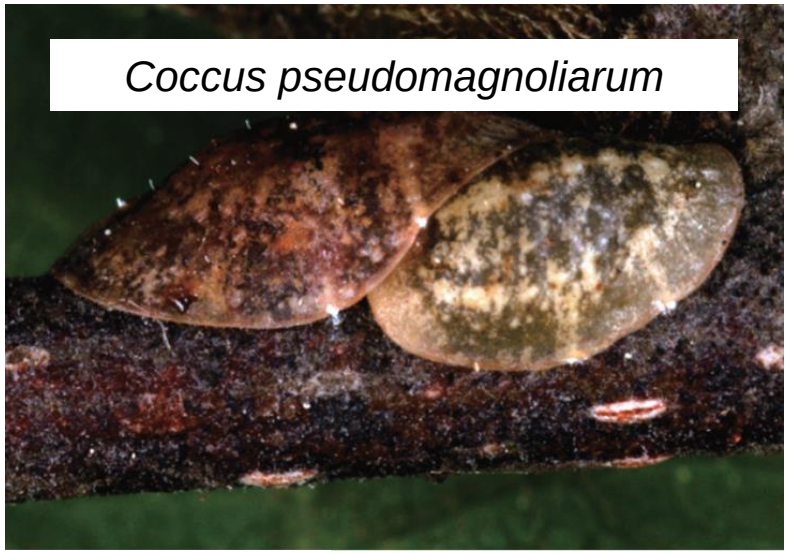
Pictures: EPPO & de a

Citrus

Eutetranychus orientalis and *E. banksii*

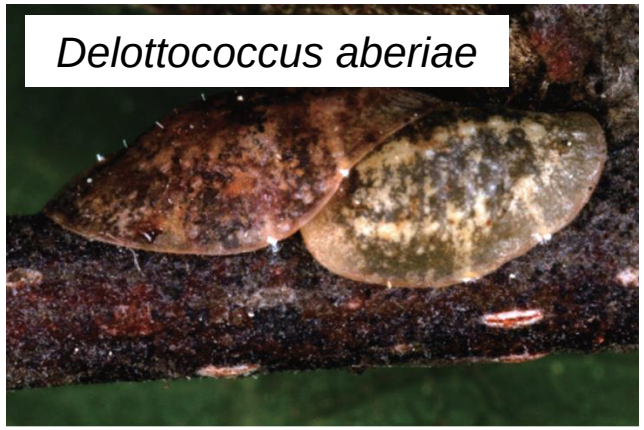


Coccus pseudomagnoliarum



Pezothrips kellyanus

Delottococcus aberiae

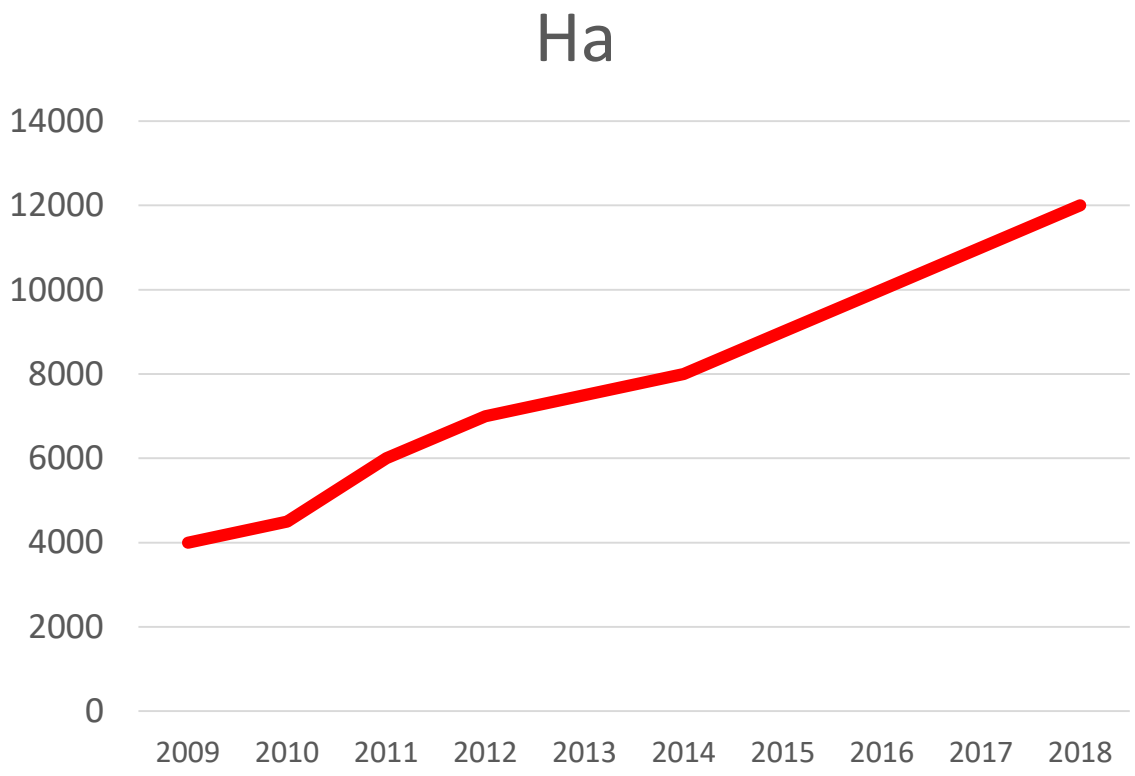


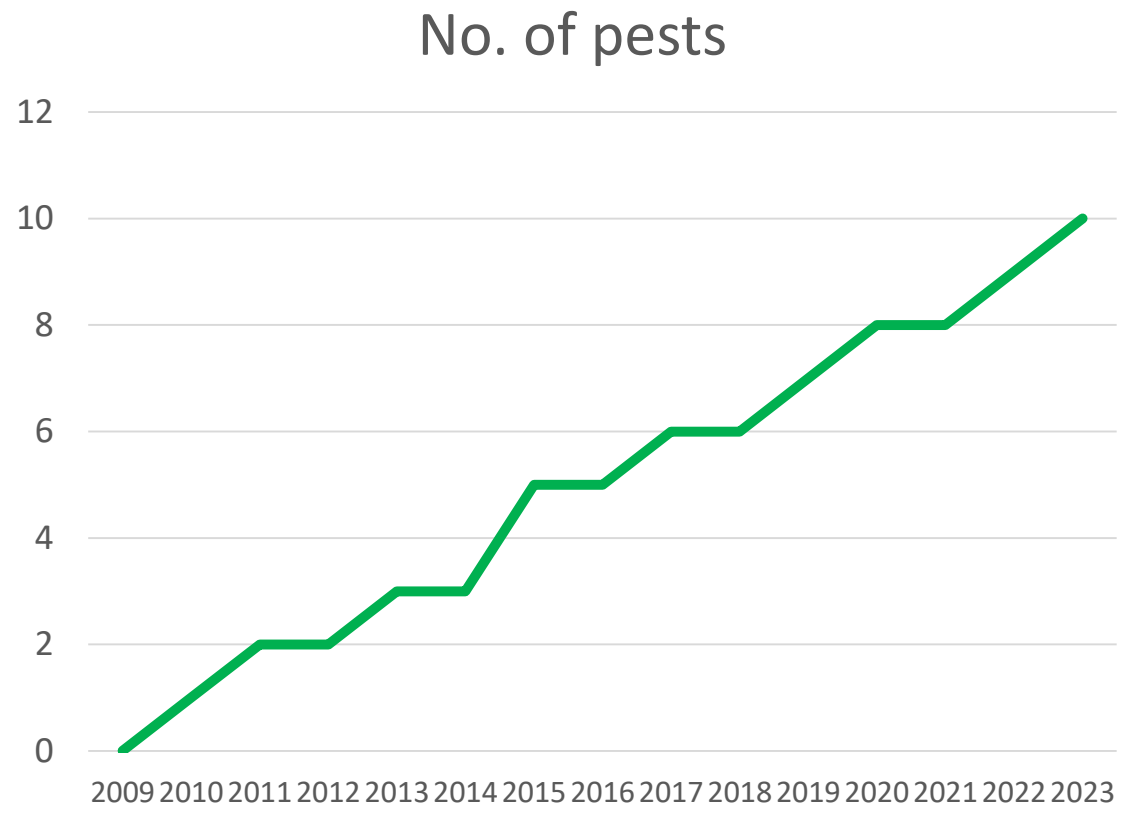
Trioza erytreae

Scirzothrips spp.

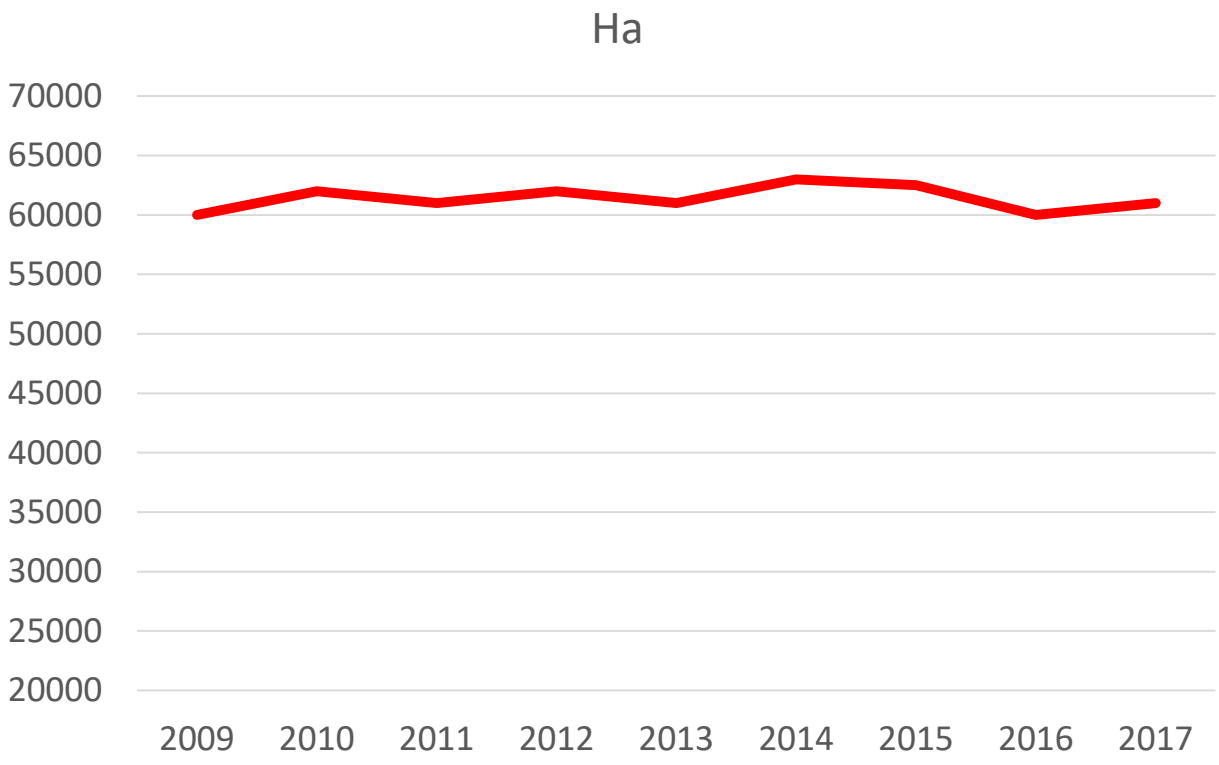
Pictures: EPPO & IVIA

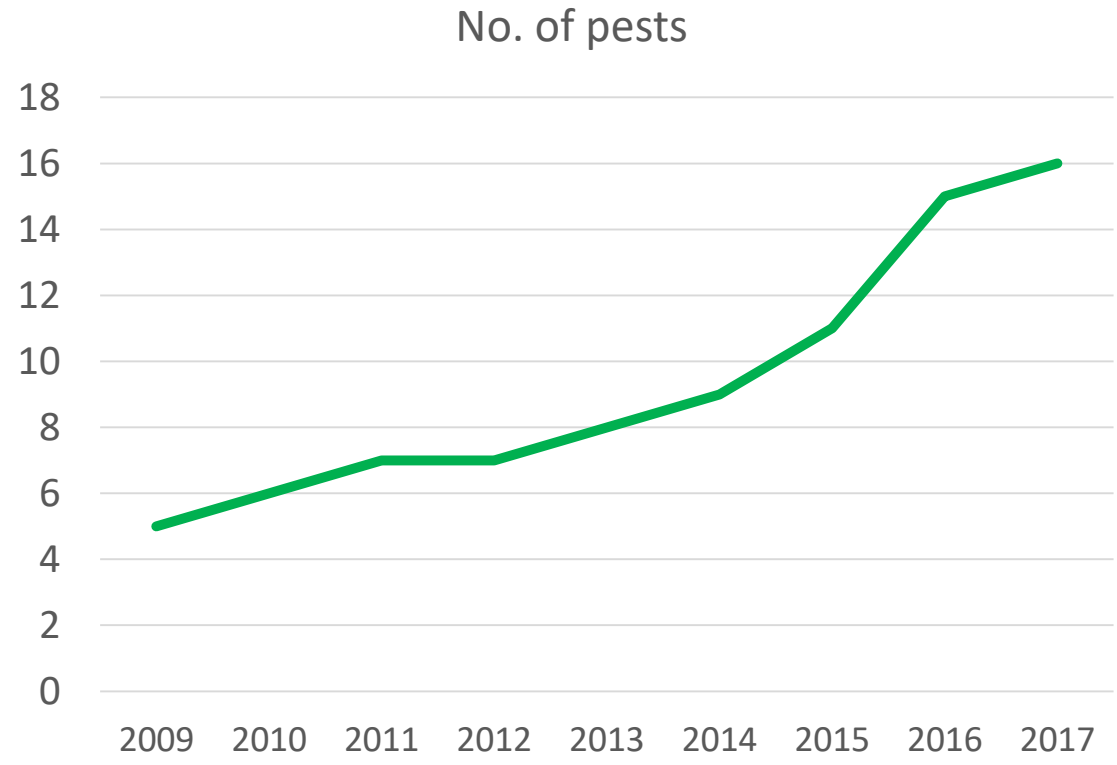
Mango


Ha
14000
12000
10000
8000
6000
4000
2000
0
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018


No. of pests
12
10
8
6
4
2
0
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023

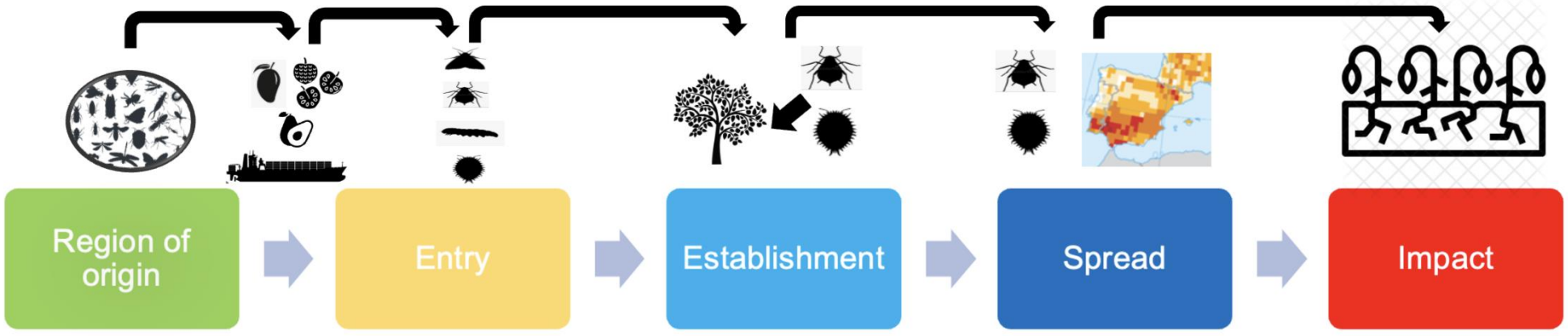
Citrus

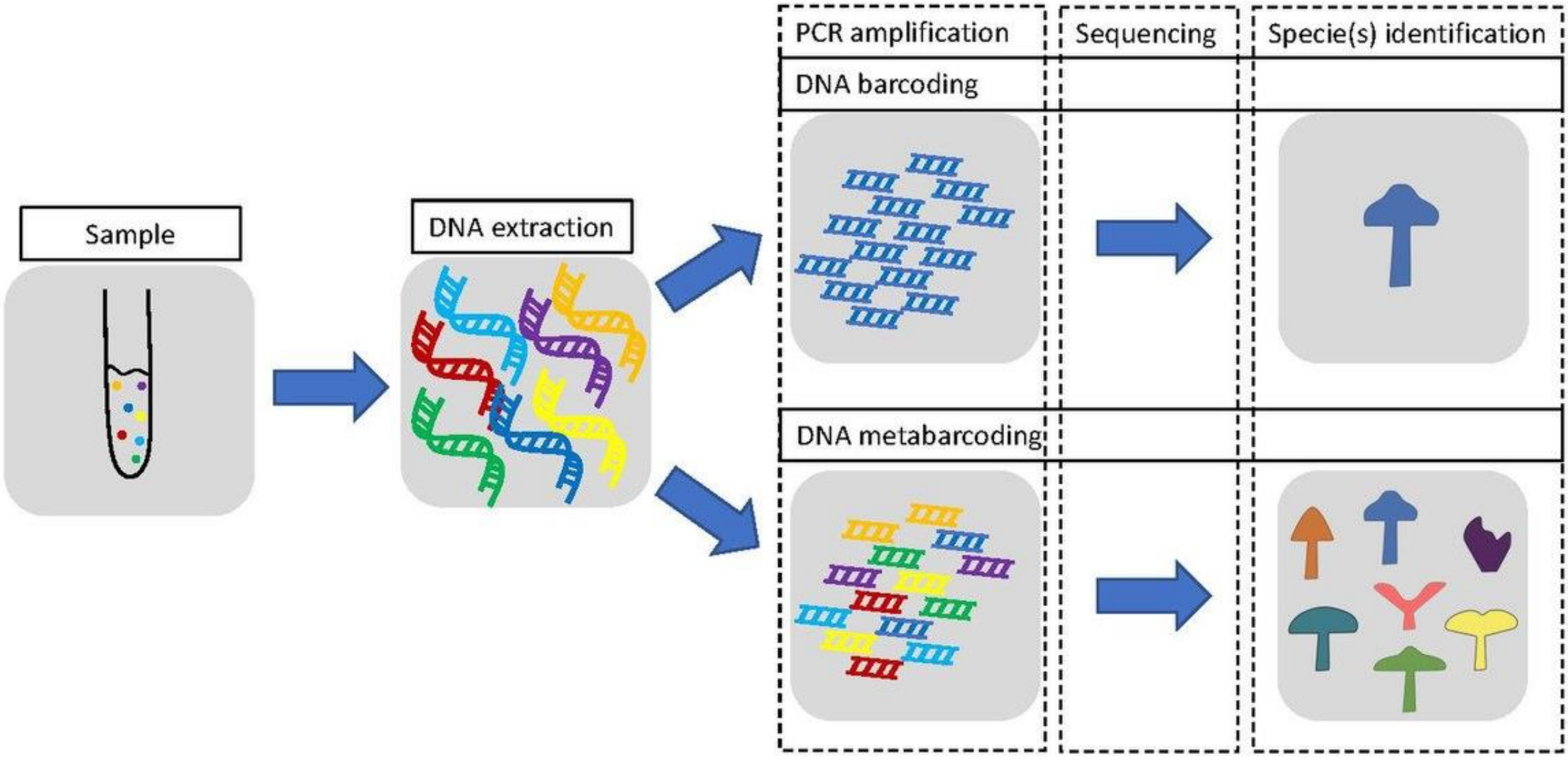

Ha
70000
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
2009
2010
2011
2012
2013
2014
2015
2016
2017

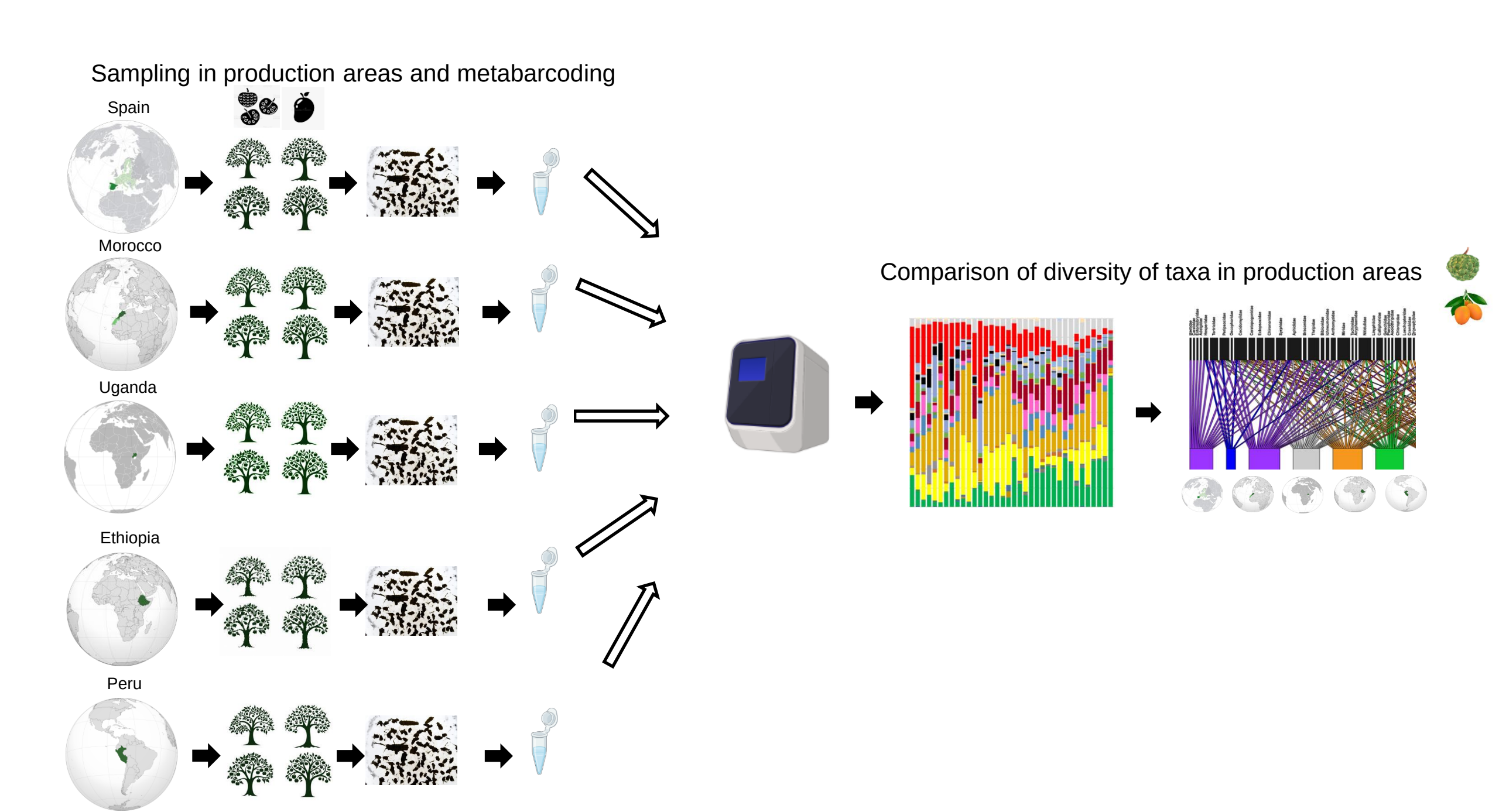

No. of pests
18
16
14
12
10
8
6
4
2
0
2009
2010
2011
2012
2013
2014
2015
2016
2017

Introduction of pests

- The rate of introduction is *ca.* 1.5 species per year.
- Spill-over effects; introduction may affect at first a “minor crop” but may jump to key native crops
- Too many introductions for NPPOs and concerning authorities to respond
- **The current situation requires new approaches**
- Prioritization and early detection
- Sampling and rapid identification


Region of origin
Entry
Establishment
Spread
Impact


Sample
DNA extraction
PCR amplification
Sequencing
Specie(s) identification
DNA barcoding
DNA metabarcoding

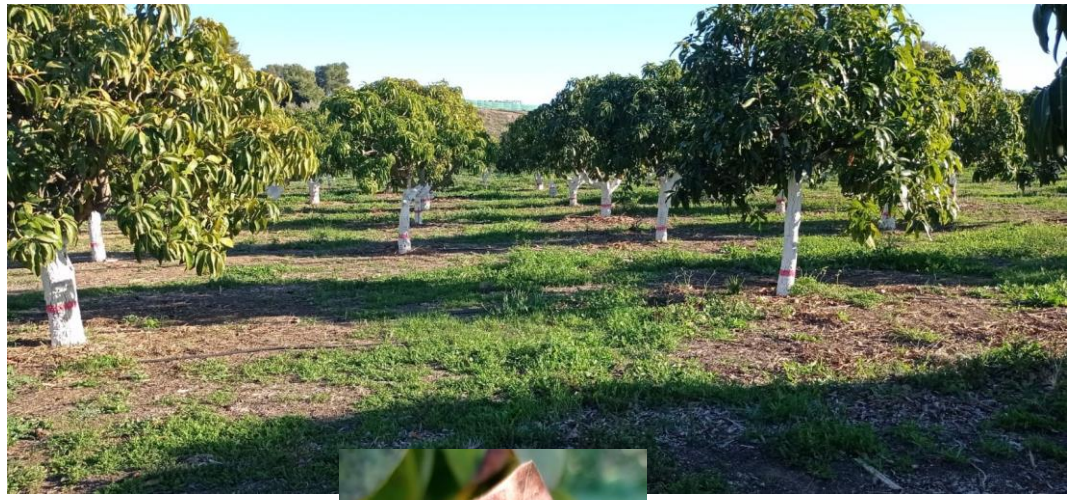

Sampling in production areas and metabarcoding
Spain
Morocco
Uganda
Ethiopia
Peru
Comparison of diversity of taxa in production areas

Questions

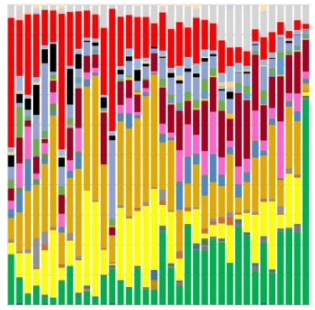
Can we use DNA barcoding for detection of regulated pests in a field situation?

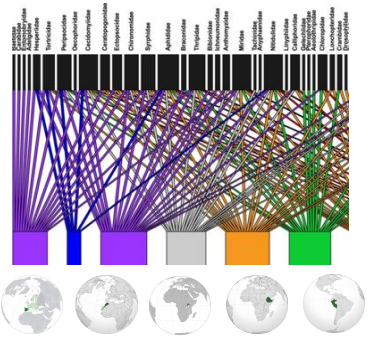
Do we detect the same targeted species using this approach?

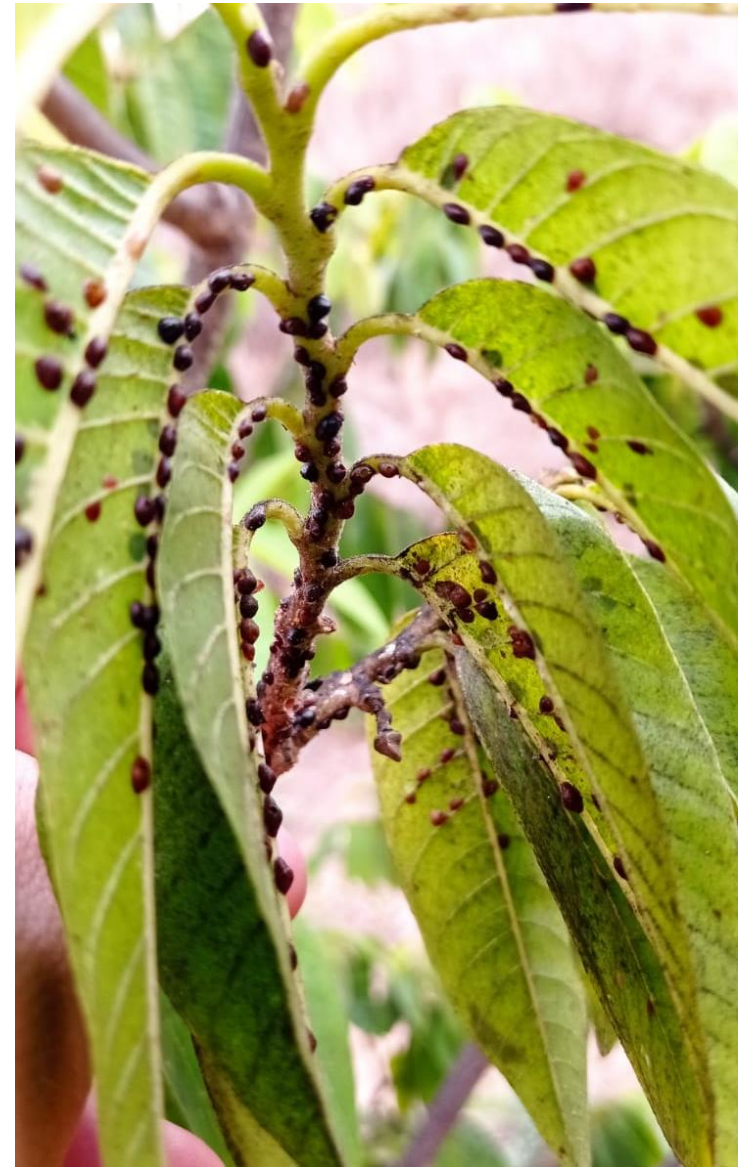
Field samplings



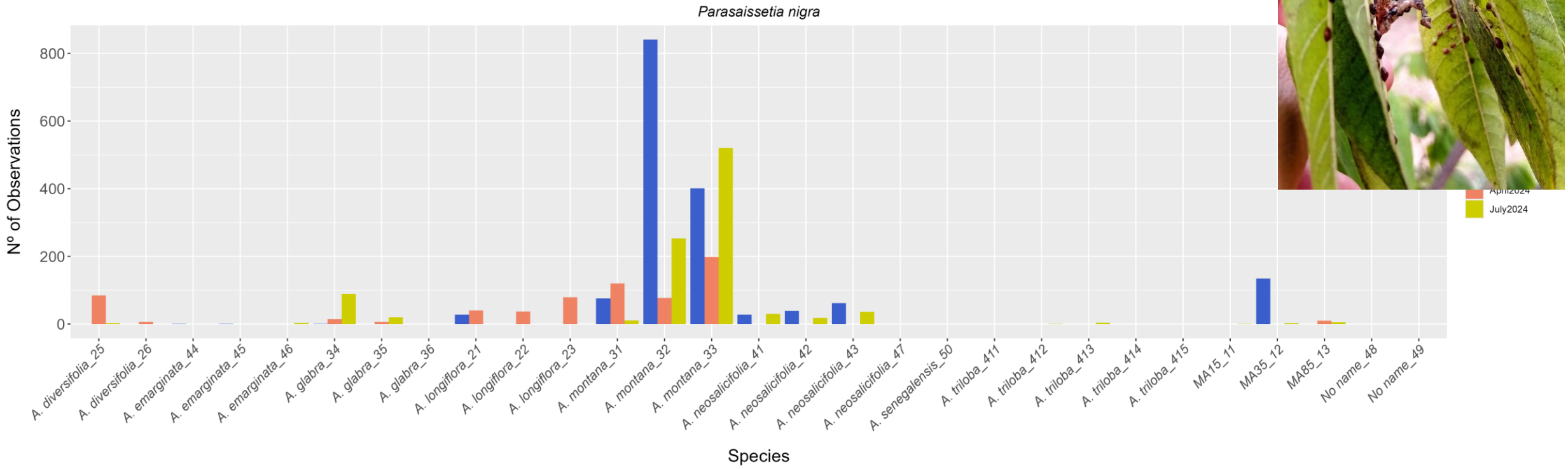
Comparison of diversity of taxa in production areas



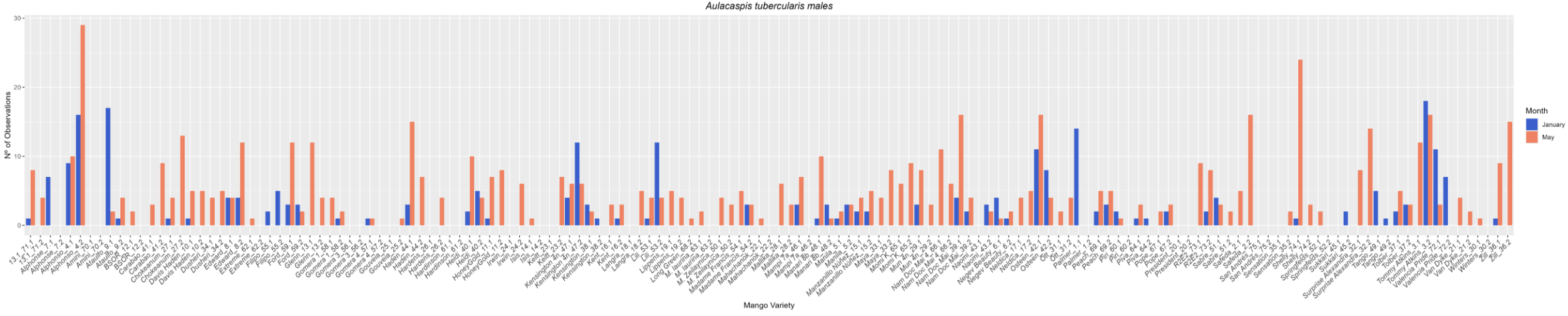




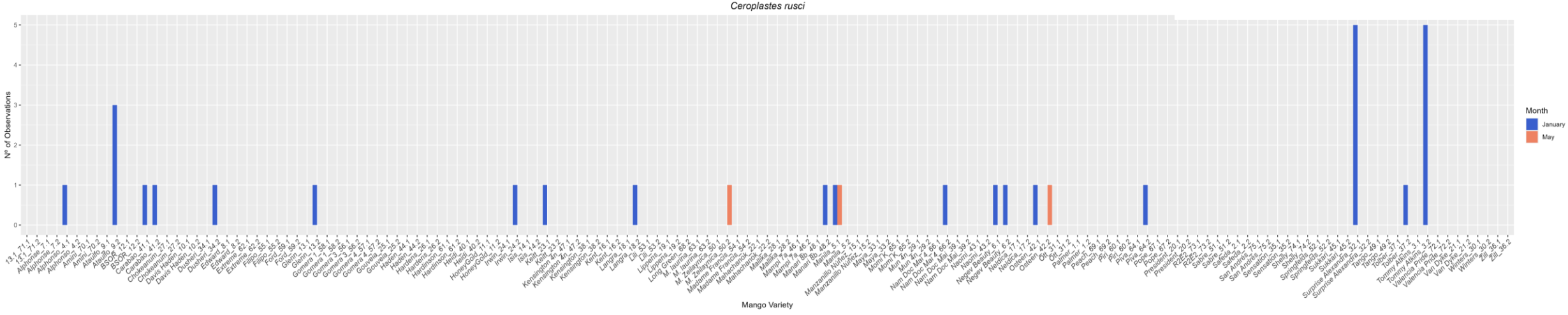
Parasaissetia nigra



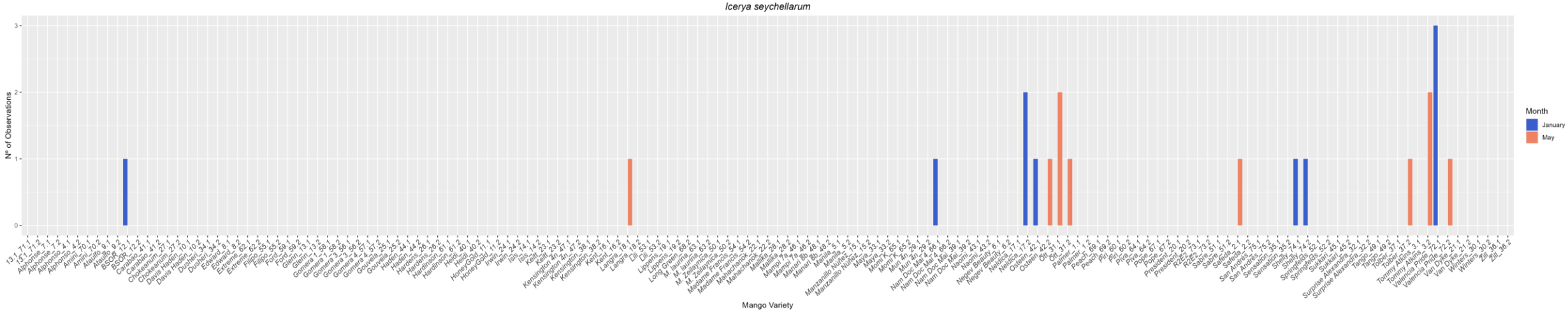
Aulacaspis tubercularis



Ceroplastes rusci



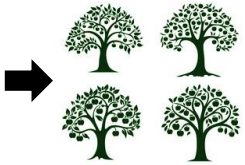
Ceroplastes rusci



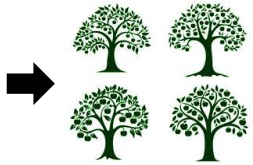
Sampling in production areas and metabarcoding

Spain

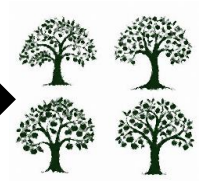
Morocco



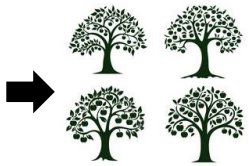
Uganda

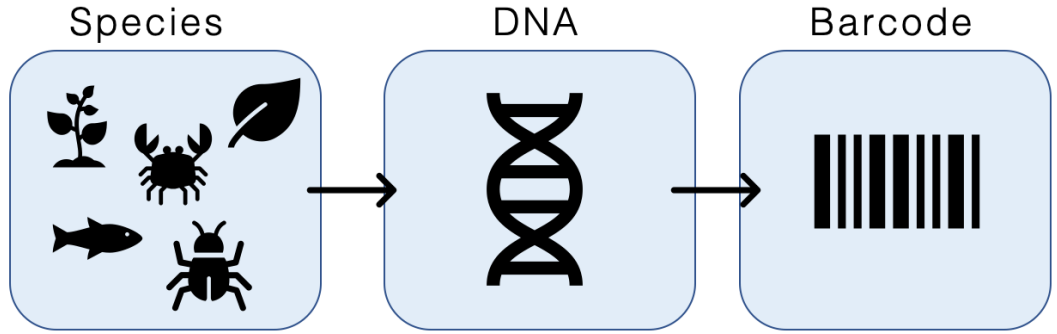


Ethiopia

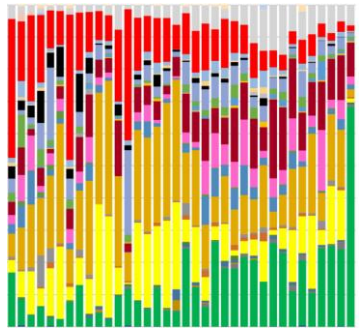


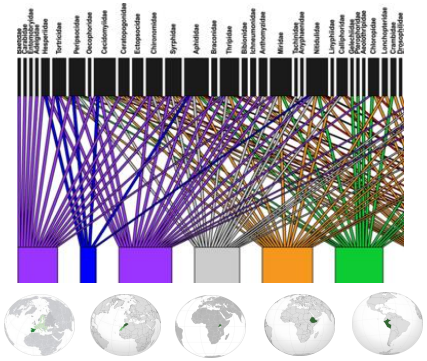
Peru

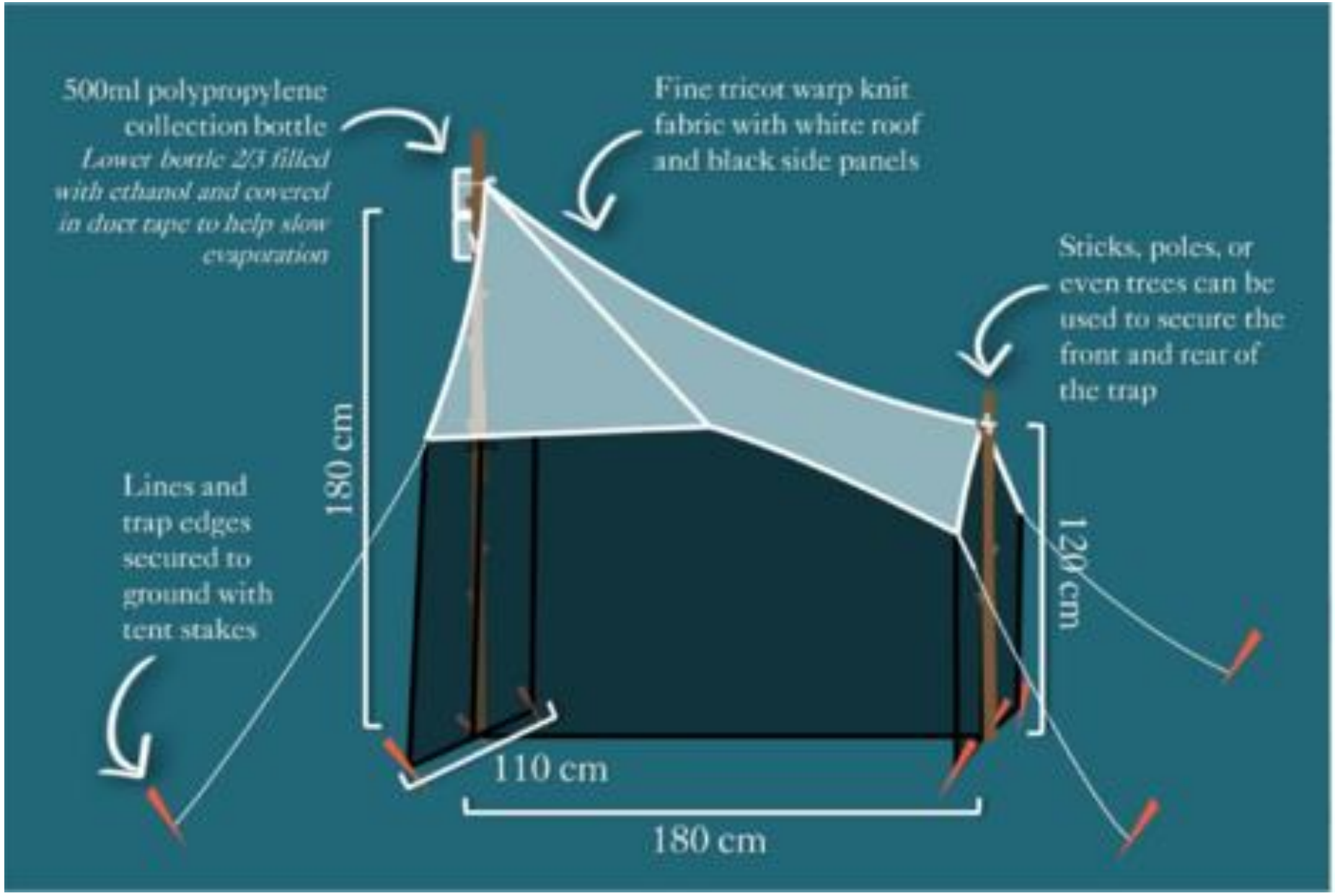




Comparison of diversity of taxa in production areas

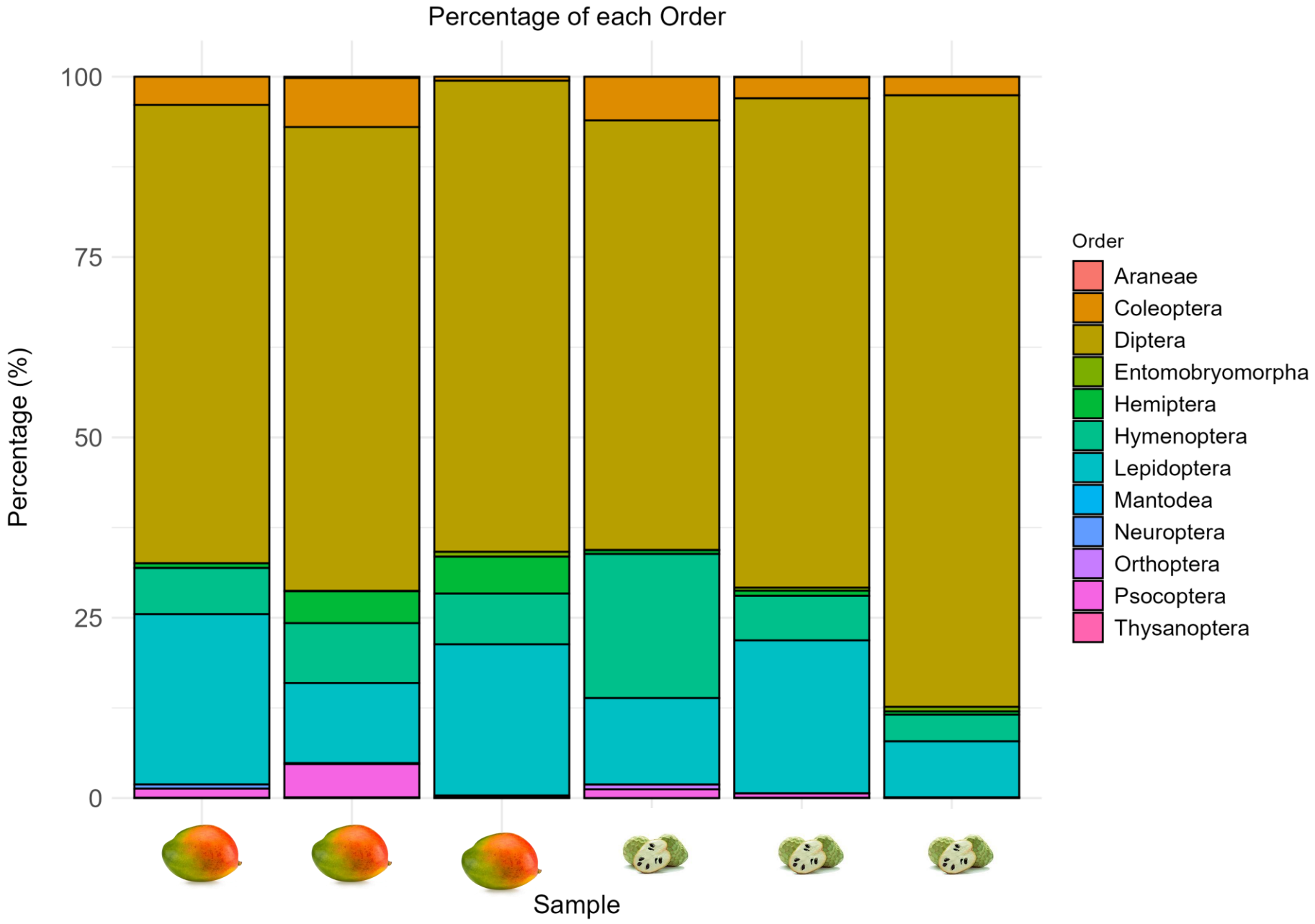


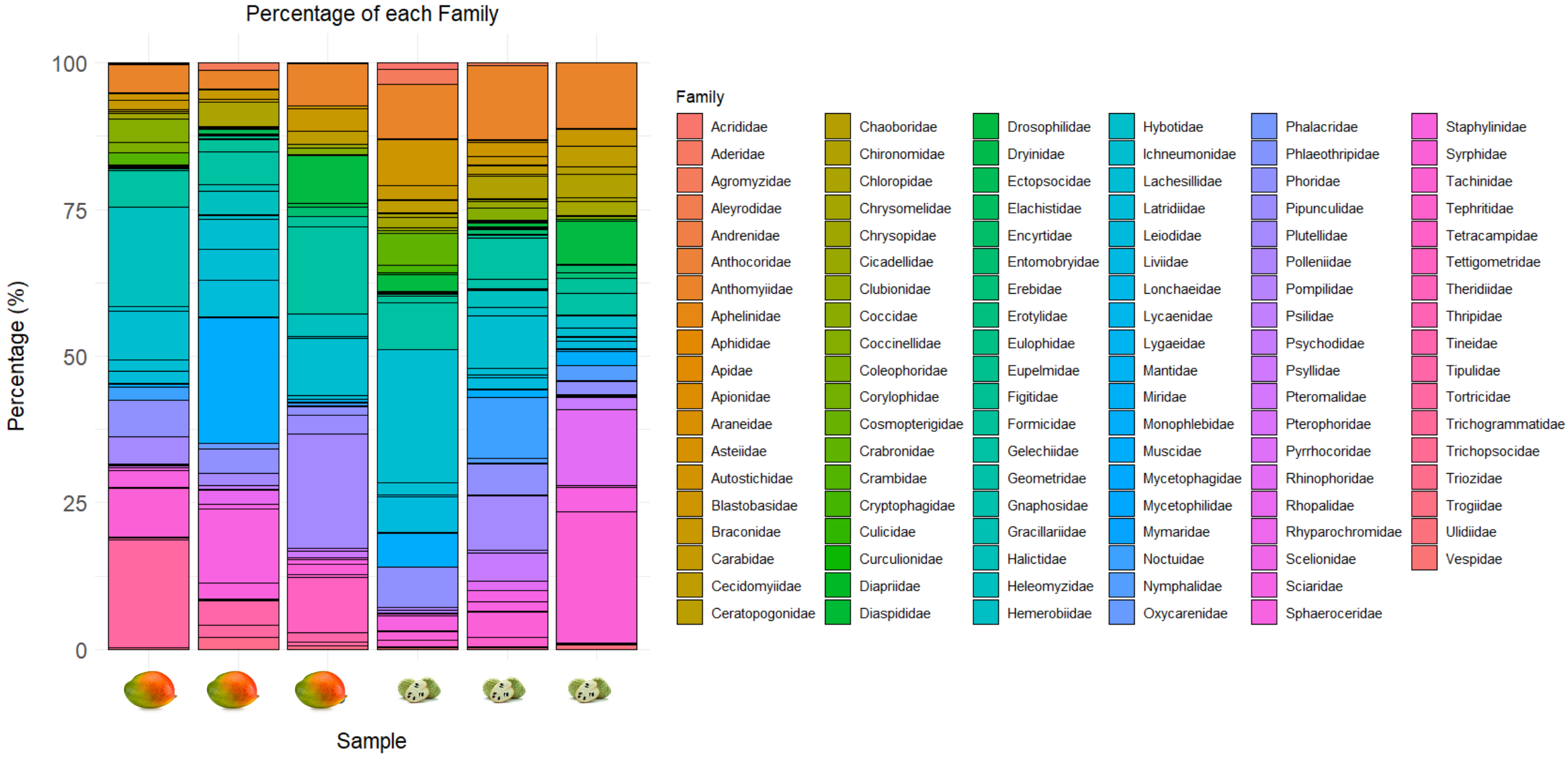


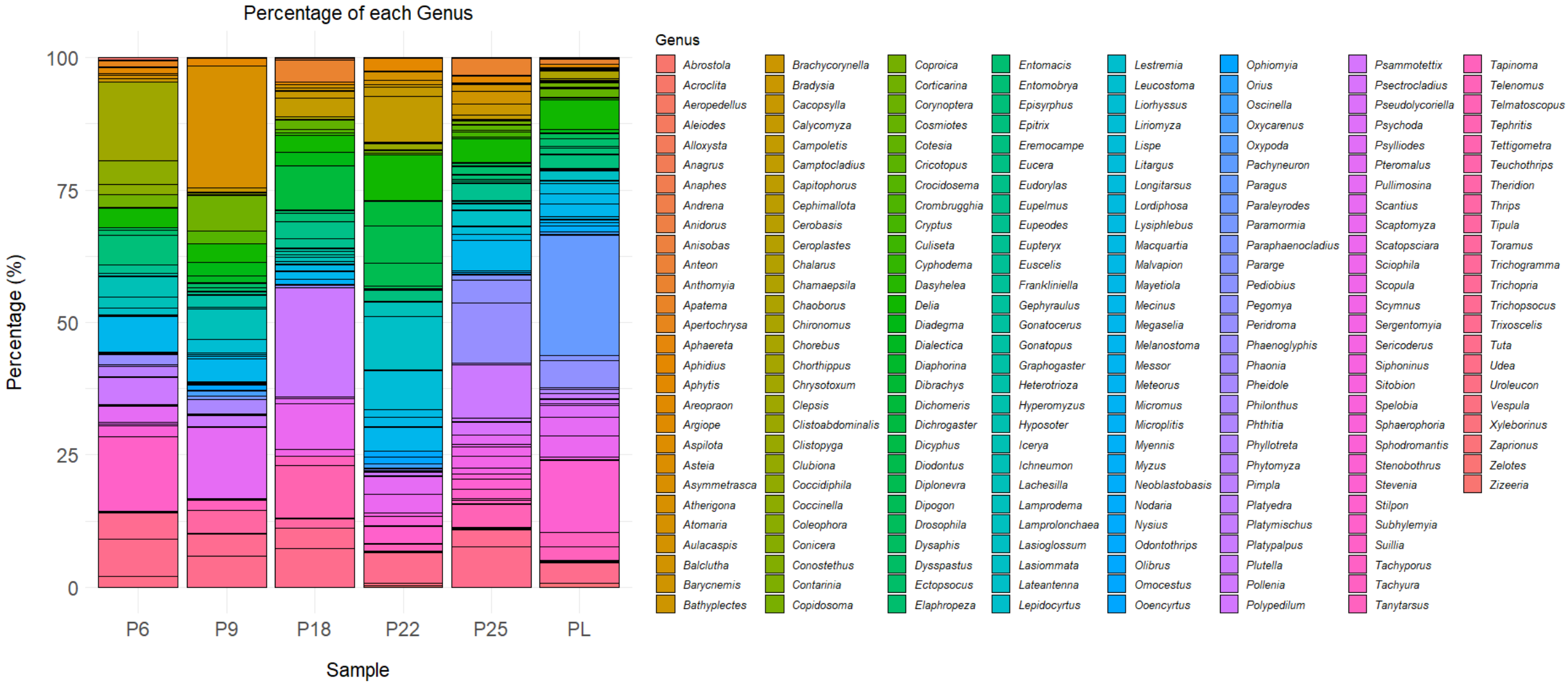

500ml polypropylene collection bottle
Lower bottle 2/3 filled with ethanol and covered in duct tape to help slow evaporation
Fine tricot warp knit fabric with white roof and black side panels
Sticks, poles, or even trees can be used to secure the front and rear of the trap
Lines and trap edges secured to ground with tent stakes
180 cm
120 cm
110 cm
180 cm

Sampling+ DNA Barcoding

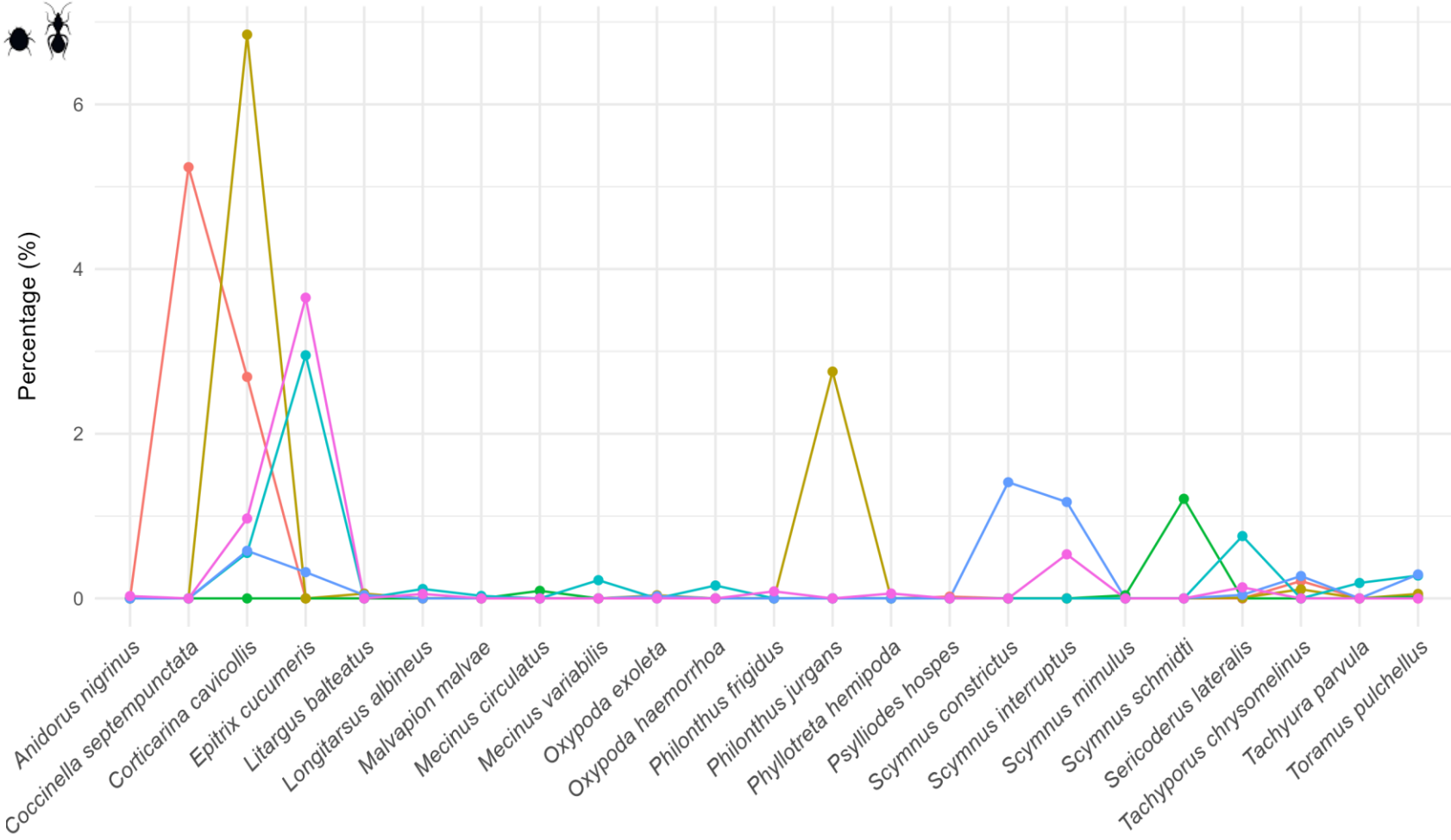
Mock samples

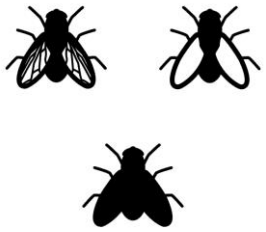

Percentage of each Order
100
75
50
25
0
Percentage (%)
Sample
Order
Araneae
Coleoptera
Diptera
Entomobryomorpha
Hemiptera
Hymenoptera
Lepidoptera
Mantodea
Neuroptera
Orthoptera
Psocoptera
Thysanoptera


Percentage of each Family
Percentage (%)
100
75
50
25
0
Sample
Family
Acrididae
Aderidae
Agromyzidae
Aleyrodidae
Andrenidae
Anthocoridae
Anthomyiidae
Aphelinidae
Aphididae
Apidae
Apionidae
Araneidae
Asteiidae
Autostichidae
Blastobasidae
Braconidae
Carabidae
Cecidomyiidae
Ceratopogonidae
Chaoboridae
Chironomidae
Chloropidae
Chrysomelidae
Chrysopidae
Cicadellidae
Clubionidae
Coccidae
Coccinellidae
Coleophoridae
Corylophidae
Cosmopterigidae
Crabronidae
Crambidae
Cryptophagidae
Culicidae
Curculionidae
Diapriidae
Diaspididae
Drosophilidae
Dryinidae
Ectopsocidae
Elachistidae
Encyrtidae
Entomobryidae
Erebidae
Erotylidae
Eulophidae
Eupelmidae
Figitidae
Formicidae
Gelechiidae
Geometridae
Gnaphosidae
Gracillariidae
Halictidae
Heleomyzidae
Hemerobiidae
Hybotidae
Ichneumonidae
Lachesillidae
Latridiidae
Leiodidae
Liviidae
Lonchaeidae
Lycaenidae
Lygaeidae
Mantidae
Miridae
Monophlebidae
Muscidae
Mycetophagidae
Mycetophilidae
Mymaridae
Noctuidae
Nymphalidae
Oxycarenidae
Phalacridae
Phlaeothripidae
Phoridae
Pipunculidae
Plutellidae
Polleniidae
Pompilidae
Psilidae
Psychodidae
Psyllidae
Pteromalidae
Pterophoridae
Pyrrhocoridae
Rhinophoridae
Rhopalidae
Rhyparochromidae
Scelionidae
Sciaridae
Sphaeroceridae
Staphylinidae
Syrphidae
Tachinidae
Tephritidae
Tetracampidae
Tettigometridae
Theridiidae
Thripidae
Tineidae
Tipulidae
Tortricidae
Trichogrammatidae
Trichopsocidae
Triozidae
Trogiidae
Ulidiidae
Vespidae

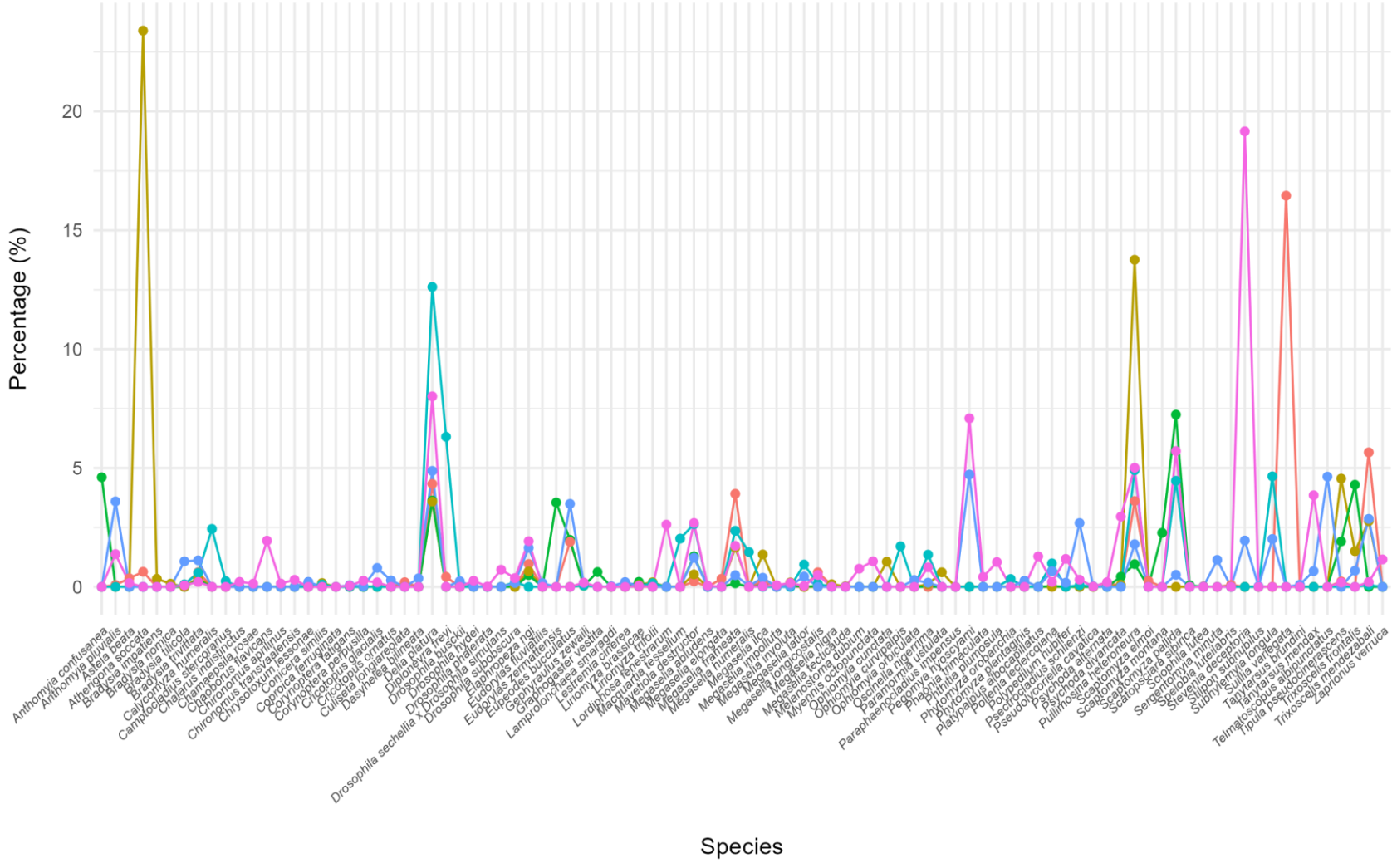

Percentage of each Genus
Percentage (%)
100
75
50
25
0
P6
P9
P18
P22
P25
PL
Sample
Genus
Abrostola
Acroclita
Aeropedellus
Aleiodes
Alloxysta
Anagrus
Anaphes
Andrena
Anidorus
Anisobas
Anteon
Anthomyia
Apatema
Apertochrysa
Aphaereta
Aphidius
Aphytis
Areopraon
Argiope
Aspilota
Asteia
Asymmetrasca
Atherigona
Atomaria
Aulacaspis
Balclutha
Barycnemis
Bathyplectes
Brachycorynella
Bradysia
Cacopsylla
Calycomyza
Campoletis
Camptocladius
Capitophorus
Cephimallota
Cerobasis
Ceroplastes
Chalarus
Chamaepsila
Chaoborus
Chironomus
Chorebus
Chorthippus
Chrysotoxum
Clepsis
Clistoabdominalis
Clistopyga
Clubiona
Coccidiphila
Coccinella
Coleophora
Conicera
Conostethus
Contarinia
Copidosoma
Coproica
Corticarina
Corynoptera
Cosmiotes
Cotesia
Cricotopus
Crocidosema
Crombrugghia
Cryptus
Culiseta
Cyphodema
Dasyhelea
Delia
Diadegma
Dialectica
Diaphorina
Dibrachys
Dichomeris
Dichrogaster
Dicyphus
Diodontus
Diplonevra
Dipogon
Drosophila
Dysaphis
Dysspastus
Ectopsocus
Elaphropeza
Entomacis
Entomobrya
Episyrphus
Epitrix
Eremocampe
Eucera
Eudorylas
Eupelmus
Eupeodes
Eupteryx
Euscelis
Frankliniella
Gephyraulus
Gonatocerus
Gonatopus
Graphogaster
Heterotrioza
Hyperomyzus
Hyposoter
Icerya
Ichneumon
Lachesilla
Lamprodema
Lamprolonchaea
Lasioglossum
Lasiommata
Lateantenna
Lepidocyrtus
Lestremia
Leucostoma
Liorhyssus
Liriomyza
Lispe
Litargus
Longitarsus
Lordiphosa
Lysiphlebus
Macquartia
Malvapion
Mayetiola
Mecinus
Megaselia
Melanostoma
Messor
Meteorus
Micromus
Microplitis
Myennis
Myzus
Neoblastobasis
Nodaria
Nysius
Odontothrips
Olibrus
Omocestus
Ooencyrtus
Ophiomyia
Orius
Oscinella
Oxycarenus
Oxypoda
Pachyneuron
Paragus
Paraleyrodes
Paramormia
Paraphaenocladius
Pararge
Pediobius
Pegomya
Peridroma
Phaenoglyphis
Phaonia
Pheidole
Philonthus
Phthitia
Phyllotreta
Phytomyza
Pimpla
Platyedra
Platymischus
Platypalpus
Plutella
Pollenia
Polypedilum
Psammotettix
Psectrocladius
Pseudolycoriella
Psychoda
Psylliodes
Pteromalus
Pullimosina
Scantius
Scaptomyza
Scatopsciara
Sciophila
Scopula
Scymnus
Sergentomyia
Sericoderus
Siphoninus
Sitobion
Spelobia
Sphaerophoria
Sphodromantis
Stenobothrus
Stevenia
Stilpon
Subhylemyia
Suillia
Tachyporus
Tachyura
Tanytarsus
Tapinoma
Telenomus
Telmatoscopus
Tephritis
Tettigometra
Teuchothrips
Theridion
Thrips
Tipula
Toramus
Trichogramma
Trichopria
Trichopsocus
Trixoscelis
Tuta
Udea
Uroleucon
Vespula
Xyleborinus
Zaprionus
Zelotes
Zizeeria

Percentage of Species by Order: Coleoptera





Percentage of Species by Order: Diptera



	Species	P18	P6	P9	P22	P25	PL	P- nigra	A- tubercularis	C. rusci	I. seychellarum	MOCK
Monophlebidae	Icerya seychellarum	0	0	0	0	0	0	0	0	0	1	1
Coccidae	Parasaissetia nigra (P)	0	0	0	0	0	0	1	0	0	0	1
Diaspididae	Aulacaspis tubercularis	0	0	0	0	0	0	0	1	0	0	1
Coccidae	Ceroplastes rusci	0	0	0	0	0	0	0	0	1	0	1

Discussion

- Use of DNA barcoding for identification was quite powerful in detecting taxa
- Groups that we were not aware of from field samplings
- We did not detect in samples from Malaise traps (and DNA barcoding) insects that we detected by visual inspection/individual tree samplings
- Need to establish detection thresholds
- Compare sampling techniques + extraction

People

- Dr. Rosario Planelló
- Helena Romero, Mónica Aquilino (UNED)
- Alemayehu Kassa
- Emiel de Meyer

Jackie Epila (Lira University, Uganda)

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