Proposta assegno A2 Junior

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Project title: Urban functional agrobiodiversity to support pollinators and human diet quality

Abstract

It is known that crops providing the greatest balance between macronutrients and micronutrients for human diet are pollinators-dependent. The risk of many diseases and metabolic disorders deriving from an unbalanced nutrients intake is increased by pollination decline, whose avoidance goes also through the provision of healthy resources by pollinators. In this framework, nutritional investigations dealing both with pollinators and human healthiness are strictly correlated and equally required. In the context of urban agriculture, this project aims at i) identifying the most important nutritional features that plant resources (i.e., pollen and nectars) may provide to pollinators to guarantee resilience to a changing environment (e.g., identification of nutraceutical compounds able to increase pollinators defenses against anthropogenic stressors); ii) evaluating whether an increasing pollination service could improve the chemical properties of fruit and seeds of some common cash crops, with particular concern for phytochemicals responsible for healthy properties (e.g., anthocyanins, lycopene, peptides).

Background, aims and significance of the proposed work

Human activity alters natural systems at different levels, ranging from the whole environment through habitat loss and fragmentation, up to the loss of biodiversity. These changes dramatically impact the ecosystem services provided to humans by biodiversity, including those related to agricultural production (e.g., pollination), regulation, health and wellbeing (Schmid et al. 2015). Among these services, mutualisms such as pollination by animals constitute elements of global importance, providing significant economic and health benefits to human society, first of all in terms of food security (Lautenbach et al. 2012; Potts et al. 2016). In the modern agriculture context, pollinators are increasingly exposed to many risks, including population decline due to the massive usage of pesticides and the loss of habitats, which diminish the availability of resources for pollinators. Wild plant nutraceutical resources such as some secondary compounds are able to induce detoxification of xenobiotics, such as agrochemicals (Mao et al., 2013), therefore the availability of a healthy diet for pollinators is of primary concern to limit their decline. Moreover, pollinators are directly responsible for up to 40% of the world's supply of some micronutrients, such as vitamin A and C, calcium, fluoride and folic acid. Insufficient intake of the key micronutrients constitutes an important risk factor for NCDs such as cardiovascular diseases, diabetes, oesophageal cancer, and lung cancer (Brittain et al., 2014; Smith et al., 2015). It is estimated that the majority of the world population is going to live in cities in the next decades. Therefore there is an urgent need to identify sustainable ways to import agriculture in the urban context by maintaining the environmental features required to guarantee the healthiness of both pollinators and human diet. Since natural habitats are increasingly fragmented and impoverished, properly designed urban green spaces and proximity urban agriculture can act as refuges for native biodiversity and particularly for pollinators. Thus, finding strategies to couple both pollinators and human health diet quality is an emerging research challenge.

Experimental plan

The project aims at improving the healthiness of urban pollinators and human diet (nutritional elements) through the improvements of plant biodiversity and agrobiodiversity. Specifically, in the context of urban agriculture, our aims is to evaluate the most charismatic plant species able to support pollinators diet quality and to evaluate the role of allogamy on the metabolic profile of fruit and seeds of common cash crops, with particular concern for micronutrients and bioactive compounds.

The project consists of 3 interconnected Work-packages ("WP").

WP1 Pollination service for enhancing micronutrients fruit content

Some studies suggested a tight relationship between fruit economic quality and cross-pollination, in contrast to self-pollination or indicated that cross-pollinated plants have higher nutritional quality, but they focus on single plant species and on macronutrients. It is not clear whether the chemical profile in terms of bioactive phytochemicals able to contrast the outbreak of NCDs or metabolic disorders are linked to allogamy or not. To shed light on these issues, in a controlled environment, the effect of density and diversity gradients of pollinators per plant on the fruit nutritional properties will be tested by exploiting some common cash crops as plant models. Briefly, a minimum of three different crop species will be exposed to at least three levels of increasing pollinator density (obtained from commercially available pollinators) and compared to control conditions (i.e. no pollinators, that is self-pollinated plants). The plant species will be chosen based on their relevance to human diet in Europe and for both being able to autonomously self-pollinate and to be cross-pollinated. Fruit deriving from these different treatments will be harvested at the same ripening condition and will be characterized for their chemical profile by the adoption of target and untarget approaches (HPLC, LC-MS/MS, GC-MS/MS and Orbitrap LC-MS) to identify specific fractions of phytocomplexes able to provide benefits for human health (e.g., lycopene, anthocyanins and peptides) in order to evaluate the influence of pollination on their properties.

WP2: Flower source and pollinators diet.

In order to improve pollinator diet and thus reverse pollinator decline (that ultimately impact on human health), pollen and nectar samples of different plant species collected in the field margins as well as from crop species will be chemically characterized to identify the most relevant plant species able to provide to pollinators a healthy diet, with particular concern for nutrients ratio and for phytochemicals responsible for defenses against pollutants and contaminants. The final aim of the investigation is to provide a list of wild species that will not be missed in a future urban-designed agriculture plan. In addition, pollinator (pollinivorous) larvae will be fed on these different kinds of pollen and beneficial outcomes in terms of chronological life-span will be tested. Both crop plants and flowering plants (the latter chosen from seed mixtures that are used for hay or green manure but also planted for the benefit of pollinators) will be tested. Pollen extracts and nectars samples will be chemically characterized by HPLC-DAD and colorimetric analyses to detect both the macronutrient content as well as the concentration of some healthy secondary compounds, such as *p*-coumaric acid, quercetin-derived metabolites and kaempherol. This WP will be carried out thanks to the collaborations with expert entomologists and pollination biologists.

WP3 Effect of phytocomplexes on human and pollinator health

The nutraceutical effects (antioxidant and immunostimulant) of defined classes of secondary metabolites extracted from the fruit and the seeds obtained in the previous WPs will be assessed by *in vitro* tests on

human cells (e.g., epithelial gastric AGS cells CRL-1739). Metabolomic analysis of cell culture extracts and cell media collected after the incubation with the antioxidant fractions of plant extracts will be performed. Cell-viability assays (MTT) and the evaluation of the anti-inflammatory activity (e.g., by measurement of IL-8 release) will be performed on the selected cell lines. These results will shed light on the impact of allogamous fruit development on the healthy properties of the final products, with inevitable consequences for human diet. Along with these analyses, the presence of reactive oxygen species (ROS) and the activity of the enzymes involved in oxidative stress reduction (glutathione reductase, glutathione peroxidase, glutathione S-transferase, catalase, superoxide dismutase) will also be tested, with the aim of assessing the effect of phytoextracts on the cells oxidative stress level. Further analyses will also evaluate if the treatment with the fruit/seeds antioxidant fractions coming from different pollination systems will differentially rescue the phenotype of cell cultures treated with oxidative stressors, such as H_2O_2, with the final aim of addressing the metabolic defences that phytocomplexes are able to trigger as a function of their different development.

Similar experiments will be conducted with *in vivo* assays on animal models (e.g., *D. melanogaster*) and to some extent on wild pollinators larvae. Such test will be performed under the supervision of Dr. A. Galimberti (UNIMIB-BTBS).

Expected results:

- Characterization of guidelines for enhancing pollinator 'health' sustainability in the framework of urban planning.

- Identification of the threshold of pollinator service in urban landscapes needed for maximizing fruit nutritional quality in terms of macro and micronutrients.

- Assessment of in vitro/in vivo phytocomplex bioactivity (e.g., immunostimulation and antioxidant).

- Comparing the bioactivity of fruit (from insect-pollinated flowers) phytoextracts in counteracting the onset and/or progression of NCDs in human.

Feasibility and financial support (single statement)

The feasibility of the project is guaranteed by active collaboration with zoologists and entomologists (A. Galimberti and P. Biella) and food chemists (L. Campone). Moreover, the department's LID platforms will allow to perform the cemical analyzes of the project.

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- 2017-2021: Sistemi Agricoli e Sviluppo Sostenibile (SASS). Ente Finanziatore: MIUR –FIRST. CUP E47F17000020009. Ruolo: Coordinatore Generale;
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PI recent papers on the topic

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