

Review

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Review

Is the Artificial Pollination of Walnut Trees with Drones Able to Minimize the Presence of *Xanthomonas arboricola* pv. *juglandis*? Review

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Abstract: Walnut (*Juglans regia* L.) is a monoecious species and although it exhibits self-compatibility, presents incomplete overlap of pollen shed and female receptivity. Thus, cross-pollination is prerequisite for optimal fruit production. Cross-pollination can occur naturally by the wind, the insects, artificially, or by hand. Pollen has been recognized as one possible pathway for *Xanthomonas arboricola* pv. *juglandis* infection, a pathogenic bacterium responsible for walnut blight disease. Other than the well-known cultural and chemical control practices, artificial pollination technologies with the use of drones could be a successful tool for walnut blight disease management of orchards. Drones may carry pollen and release it over crops or mimic the action of bees or other pollinators. Although this new pollination technology could be regarded as a promising tool, pollen germination and knowledge of pollen as a potential pathway for the dissemination of bacterial diseases remain crucial information for the development and production of aerial pollinator robots for walnut trees. Thus, our purpose was to describe a pollination model with fundamental components, the identification of the “core” pollen microbiota, specify an appropriate flower pollination algorithm, design an autonomous precision pollination robot, and minimize the average errors of flower pollination algorithm parameters through machine learning and meta-heuristic algorithms.

Keywords: cross pollination; *Juglans regia*; literature review; self-compatibility; walnut blight disease

1. Introduction

The process of pollination involves the transfer of pollen grains from the anther, the male portion of a flower, to the female part (stigma) of the same or another plant. There are two types of pollination form: i) self-pollination (autogamy), in which pollen is deposited on the stigma of the same or another flower on the same plant, and ii) cross-pollination (allogamy), in which pollen is transferred from one plant to the flower of a genetically different plant or cultivar. Walnut trees are self-compatible, but they require cross-pollination with another walnut tree to produce nuts due to protandrous, meaning that the male flowers mature – release pollen before the female flowers become receptive, and pollen shedding occurs before female bloom begins or protogynous, meaning that the female flower begins opening – become receptive - prior to the pollen shedding. Cross-pollination involves transferring pollen from the male flowers of one walnut tree to the female flowers of another. This can occur naturally, with the help of the wind (wind-blown pollen), or insects such as bees, or artificially, through the process of hand pollination [1].

On walnut trees, the pollen is produced in catkins which may also be colonized with *Xanthomonas arboricola* pv. *juglandis*, the caused agent of walnut blight disease [2,3]. Mainly the disease can affect the leaves, stems, and nuts of the tree, leading to reduced yield and poor-quality nuts. *Xanthomonas arboricola* pv. *juglandis* is primarily spread by water, either through rain or irrigation, and can be more severe during warm and wet weather [4,5].

Once the bacterium enters the tree tissues, it can survive and spread within the tree through the sap. To prevent and control *X. arboricola* pv. *juglandis*, farmers can use a combination of cultural and chemical control measures. This may include removing infected plant material, managing irrigation practices to reduce water on the foliage, and applying copper-based fungicides during the dormant season or at the first signs of infection. Here, it is important to note that the overuse of chemical control measures can lead to the development of resistant strains of the bacterium, so it is important to use a holistic approach to disease management that incorporates a variety of methods. Additionally, early detection and rapid response to infected trees can help limit the spread of the disease to other trees in the orchard.

Research has shown that inoculum of *X. arboricola* pv. *juglandis* is also disseminated through pollen [3,6]. Those evidence-based early detections of developmental-behavioral problems in primary infection of *X. arboricola* pv. *juglandis* on plant symptomless materials such as catkins and pollen besides the symptomatic materials (leaves and fruits and other plant debris) should be considered.

Pathogenic bacteria and pollen are two distinct biological entities that are not directly related to each other. While pathogenic bacteria and pollen are not directly related, they can sometimes interact in certain situations. For example, pollen can sometimes serve as a carrier for pathogenic bacteria. Pollen of *Actinidia* spp., is a good substrate for *Pseudomonas syringae* pv. *actinidiae* colonization and survival and as a pathway for its spread [7]. Therefore, pollen may be a conduit for the spread of *P. syringae* pv. *actinidiae* and kiwifruit bacterial canker. [7]. Further, pollen dispersal has been presented for *Erwinia amylovora* of pome fruits [8], and for *X. arboricola* pv. *juglandis* of walnut [9,10]. Moreover, pollen was recognized as a possible pathway for *X. arboricola* pv. *juglandis*, although further studies were suggested to confirm if pollen could spread the pathogen [3]. Viable pathogenic bacterium such as *X. arboricola* pv. *juglandis* were often isolated from pollen and infected pollen also represents a potential route of introduction into healthy walnut groves even in case of mechanical pollination [3,6]. Considering walnut cv. Chandler and cv. Serr results showed that pollen was recognized as a possible pathway for *X. arboricola* pv. *juglandis* [3].

Therefore, the survival of the bacterium *X. arboricola* pv. *juglandis* in catkin buds and catkins should be checked before the bacterium overwinters in blighted leaf and catkin buds. When water carries the bacteria to the leaves and stigmatic surfaces of the flowers, the blight spreads from these reservoirs. When catkins open and staminate flowers release pollen, the disease may also spread through the air. Contaminated pollen may be carried by air winds and deposited on any young growing parts of the tree [4].

In our view, these parameters are important and will be further analyzed in this review paper. Further, we will try to answer what are the advantages of pollination drones and how they can help to prevent or reduce the risk of the walnut blight disease. In the end, we have designed a path-planning algorithm for a pollination robot that involves determining the disease inoculum and an optimal route for the robot to pollinate flowers efficiently.

2. Walnut Blight Prevention

Walnut Blight and Conditions

Typically, the cycles of walnut blight bacteria are dependent upon weather conditions and rainfall during the growing season. Infrequent rainfall during the spring may lead to monocyclic progress, while frequent spring rainfall tends to favor polycyclic disease epidemics. Rainfalls during late spring (after leaf growth) have been reported to favor the spread of the *X. arboricola* pv. *juglandis* bacterium, which causes serious damage to trees, and it is responsible for significant crop losses, which can reach more than 50% of nut drops [11].

All aerial walnut organs, including as catkins, female flowers, leaves, and fruit, are infected [3]. Necrotic lesions on the fruit, twigs, and foliage are characteristic disease symptoms. Leaf lesions consist of small water-soaked spots, surrounded by chlorotic halos that extend to become brown necrotic lesions. Fruit lesions begin as tiny, water-soaked spots and develop into pericarp and inner

nut tissue necrosis, which results in early fruit drop. After shell hardening, infections often only impact the epicarp [12].

Cankers serve as a source of inoculum for leaves and nutlet infections [4,13,14]. Populations of *X. arboricola* pv. *juglandis* found on dormant buds serve as the primary inoculum for nut infections [13]. Tissues that have recently been infected can as secondary sources of inoculum for the pathogen. Pollen released from infected catkins plays a role in pathogen dissemination [2,13]. The bacterium is transmitted through moisture, particularly through the combined action of wind and rain [15]. The cultivars Chandler and Vina exhibit a significant susceptibility to *X. arboricola* pv. *juglandis*, thus demonstrating the potential of these cultivars to be infected with *X. arboricola* pv. *juglandis* under conditions favorable for the disease [5,13,16]. The cultivars terminal fruit fulness cultivars Milotai, Marbot, Sibisel, and Ronde de Montignac and the lateral fruit fulness cultivars Chandler, Sunland, and Techama were found to be highly susceptible cultivars, making it possible to serve as a host response to bacterial blight infections at different leaf and fruit growth stages [12].

Overall, the degree of infection caused by *X. arboricola* pv. *juglandis* bacterium depends on: a) the quantity of the pathogen presented in individual catkin buds and catkins (inoculum), b) the quantity of walnut blight cankers present on certain walnut varieties, c) the environmental conditions such as rain, which plays a significant role in spreading bacteria and aiding infection and d) the early leafing varieties that are most severely affected.

Besides the favorable conditions for the disease, research suggests that pollen released from infected catkins plays a role in pathogen dissemination [13,14]. Aerial dissemination of infected pollen from diseased catkins may also transmit the bacterium, *X. arboricola* pv. *juglandis*, to pistil-late flowers. However, this source of inoculum might be region specific [17] or by different origin of the propagation material [18]. Up today new evidence, provides data that infections depend on pollen, especially in walnut orchards with varieties where catkins emerge before the pistillate flowers i.e., cv. Chandler. Indeed, pollen is important for spreading bacteria and aiding infection [3]. Even more, the isolation of *X. arboricola* pv. *juglandis*, in late winter-early spring led to the finding that primary inoculum is present in buds (overwintering), catkins, and female flowers [4,13].

So, it's crucial to select walnut varieties resistant to bacterial blight and to implement good orchard management techniques, such as pruning and fertilization to keep trees healthy and less susceptible to disease. Moreover, one should be aware of pollen released from infected catkins, which contributes to the spread of the pathogen *X. arboricola* pv. *juglandis*. If bacterial blight is present in the orchard, it may be necessary to apply fungicides or use other treatments to prevent the disease from spreading, such as artificial techniques for cross-pollination, which probably has a direct cause or prevent bacterial blight in walnut trees. Apart from the infected catkins with *X. arboricola* pv. *juglandis* which plays a role in pathogen dissemination, walnuts produce pollen that is desiccation intolerant. Pollen that do not have homeostatic mechanisms for maintaining a constant water content also die rapidly after opening of the anther or after pollen dispersal [19]. The previously described 'partially hydrated' or, more precisely, desiccation sensitive pollen of this type may have served as the connection point with *X. arboricola* pv. *juglandis* when the environmental conditions such as temperature, and relative humidity, can affect pollination processes or are highly favorable for the pathogen growth in plants [19].

However, this pollen's property related to walnut blight disease during the flower cycle when the daily temperature and leaf wetness are more favorable for *X. arboricola* pv. *juglandis* has not been supported with detailed experimental data. The epiphytic colonization of the stigmas of the kiwifruit flower, inoculated by pollen contaminated with the GFPuv-labelled *Pseudomonas syringae* pv. *actinidiae* (Psa), responsible for the bacterial canker of the kiwifruit, concluded that the Psa, is often transmitted to the stigma by pollen contamination [20]. Further, it is well known that plants employ sexual mimicry, and flowers mimic the mating signals of their pollinator insects. Based on the mimicry phenomenon, researchers demonstrated that fire blight, a serious disease of apple and pear trees, requires the combination of open blossoms, warm temperatures, and wet weather. The disease spreads rapidly from flower to flower by wind-driven or splashing moisture and by pollinating bees or other insects. So, the specific mimic odors which serve as a tool to enhance honeybee foraging and

pollination activities in pear and apple crops spread serious diseases such as a fire blight of pear and apple trees [21,22]. So, concerning the walnut blight disease, fundamental questions are: is flower/corolla closure linked to a decrease in viability of desiccation-sensitive pollen? Is this disease related to other pollination activities or phenomena such as mimicry or dissemination by the wind?

As far as walnut plants it concerns, if pollen is served as a possible pathway for the dissemination of *X. arboricola* pv. *juglandis* and walnut blight disease, to prevent the disease from spreading, apart fungicides it is necessary to use artificial techniques for cross-pollination as mentioned above with uninfected pollen (pollen that is free from the above pathogen or diseases).

3. Walnut buds: bloom, and pollination events - Cross-Pollination

Walnuts are monoecious and have male and female flowers on the same tree. Male flowers are formed in structures called catkins. They developed directly on the prior year's growth and are easy to identify by eye. At leaf out, the preformed shoot develops, and compound leaves start to grow before the pistillate (female) flowers form. That's explains why walnut cultivars are indicated by both their leaf out date and bloom date. Female flowers are only receptive to pollination for a short time. In particular, pollen shed from staminate (male) flowers during anthesis is spread by wind (wind-blown pollen), and remains viable for a short time, generally up to 48 hours. As already mentioned, walnut is self-compatible but have adapted a mechanism called dichogamy to reduce the degree of inbreeding. Walnuts are classified as heterodichogamous, however, the majority of commercial walnut cultivars such as Chandler and Serr are protandrous, meaning that the male flowers mature and pollen shed occurs before the female bloom begins. So protandrous cultivars i.e. Serr may be a large contributor to protogynous cultivar pollination. In most walnut-growing regions, dichogamy is the reason for including pollinizers in orchards. Moreover, in certain cultivars such as Serr, an excessive of pollen on pistillate flowers (too much pollen), has been identified to be the cause of pistillate flower abortion [1].

During cross-pollination, pollen is transferred from the male reproductive organ (stamen) of one plant to the female reproductive organ (pistil) of another plant of the same species, resulting in fertilization and the production of seeds. In the case of nut trees like walnuts, cross-pollination is necessary for the trees to produce nuts. Cross-pollination can occur naturally through the action of wind or insects such as bees, or it can be done artificially through hand pollination, in which pollen is manually transferred from one tree to another. Natural cross-pollination can be affected by factors such as the proximity of the trees, the timing of flowering, and the presence of pollinators.

Walnuts, require cross-pollination for optimal nut production. Cross-pollination increases the genetic diversity of the trees and can lead to larger and more flavorful fruit. However, not all nut tree varieties are compatible for cross-pollination. It's important to choose compatible varieties and to plant them close enough for natural cross-pollination to occur. In some cases, artificial pollination may be necessary to ensure adequate pollination and fruit set. This is especially true in orchards where natural pollinators are scarce or when the weather is unfavorable for pollination. Artificial pollination involves manually transferring pollen from the male flowers of one tree to the female flowers of another. As mentioned above walnut trees are probably requiring cross-pollination with another walnut tree to produce nuts and minimize the bacterial risk contamination. This can occur naturally, or artificially with air vehicles, or through the process of hand pollination or design specific aerial pollination systems for walnut trees.

4. Artificial Pollination - Artificial Pollination Technology

Pollination is critical for many crops for successful production. To avoid pollination failure by weather events or bloom asynchrony on both insects, and wind-based pollination systems, plants require artificial pollination systems that can provide the security of yield crops [23].

There are various reasons for resorting to artificial pollination, including:

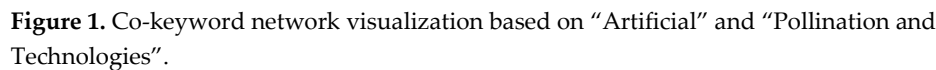
1. Crop Yield Enhancement: In agriculture, artificial pollination is sometimes employed to increase crop yields. This can be particularly important for crops where natural pollination may be insufficient [24].

2. Control of Pollination: Artificial pollination allows for precise control over the pollination process. This is useful in hybrid seed production, where specific traits can be selected [25].
3. Overcoming Pollination Challenges: Some crops may face challenges with natural pollination due to factors like low insect activity, unfavorable weather conditions i.e., wind, or geographic isolation. Artificial pollination can overcome these challenges [26].
4. Biotechnological Research: In scientific research, artificial pollination can be used to study plant genetics, breeding, and other aspects of plant biology [27].

Artificial pollination technology involves various methods and tools designed to facilitate the pollination process in plants by assisting the natural transfer of pollen. Some of the technologies and techniques used for artificial pollination include:

1. Drones: Unmanned aerial vehicles (UAVs), or drones, equipped with special devices can be used for pollination. These devices may carry pollen and release it over crops i.e., walnut trees, or mimicking the action of bees or other pollinators [28].
2. Robotic Pollinators: Small robots designed to mimic the behavior of natural pollinators can navigate through crops, transferring pollen between flowers. These robots are often equipped with cameras and sensors to identify and locate flowers [29].
3. Spraying Devices: Some systems involve the use of sprayers to disperse pollen over crops. These devices can be mounted on tractors or other vehicles, releasing pollen in a controlled manner [30].
4. Electrostatic Pollination: This method uses an electrostatic charge to adhere pollen to flowers. The charged pollen is attracted to the stigma, increasing the chances of successful pollination [31].
5. Vibrational Devices: Certain crops respond well to vibrational stimulation, which can be achieved through devices that vibrate the flowers, causing the release of pollen [32].
6. Artificial Flowers: In controlled environments like greenhouses, artificial flowers containing pollen can be placed strategically to enhance pollination [33].
7. Automated Pollination Systems: Some systems use automated robotic arms or other mechanical devices to transfer pollen between flowers. These systems can be programmed to work efficiently and quickly [34].

The development of artificial pollination technologies is driven by the need to address challenges such as declining bee populations, environmental factors and the increasing demand for efficient and reliable pollination in agriculture. By using the VOS viewer mapping software and the Scopus bibliographic database based on the search strategy criteria “Artificial and Pollination and Technologies” (Figure 1), the above mentioned technologies show promise but they are still in the early stages of development (Co-keyword: artificial intelligence, Figure 1), and may vary in effectiveness (Co-keyword: optimization, Figure 1), depending on the specific crop, environmental conditions and probably other parameters such as the disease inoculum [35–39].

[illegible]

The keywords for “Artificial” and “Pollination and Technologies and Walnut” were presented as 13 clusters defined by 310 keywords (items), which contributed a total 100%, as presented in Figure 2.

Cluster 1 (Figure 3), is defined by 52 keywords (items), with keywords including “matlab”, “adams matlab cosimulation”, “computational fluid dynamics”, “cosimulation”, “flight simulators”, “flying robots”, “quad rotors”, “aerial robot”, “robotics”, “agricultural robots”, “system stability”, “vibration analysis”, “vibration transmissibility”, “software testing”, “antennas”, “artificial pollinations”, “fruit production”, “population growth”, “population statistics”, “walnut pollinator”, “crop growth”, “wind disturbance”, etc.

Cluster 2 (Figure 4), is defined by 45 keywords (items), with keywords including “algorithms”, “bacterial artificial chromosome”, “chromosomes, artificial, bacterial”, “contig mapping”, “expressed sequence tags”, “genome analysis”, “genotyping techniques”, “heterozygosity”, “homozygosity”, “physical map”, “plant genome”, “plant growth”, “pollen sources”, “population genetic structure”, “sequence analysis, dna”, “single nucleotide polymorphism”, “vegetative propagation”, etc.

Based on the above observations, cluster 1 and 2 (Figures 3 and 4), “Artificial Pollination Technologies” include knowledge of: a) mechanical pollination technology i.e., aerial vehicles (UAVs), robotic and autonomous pollinators (hardware), and basic simulation methodologies for controlling a quadrotor (software). b) Mother nature pollination technology i.e., pollen (pollen germination), crop (cultivars), microorganisms (bacteria).

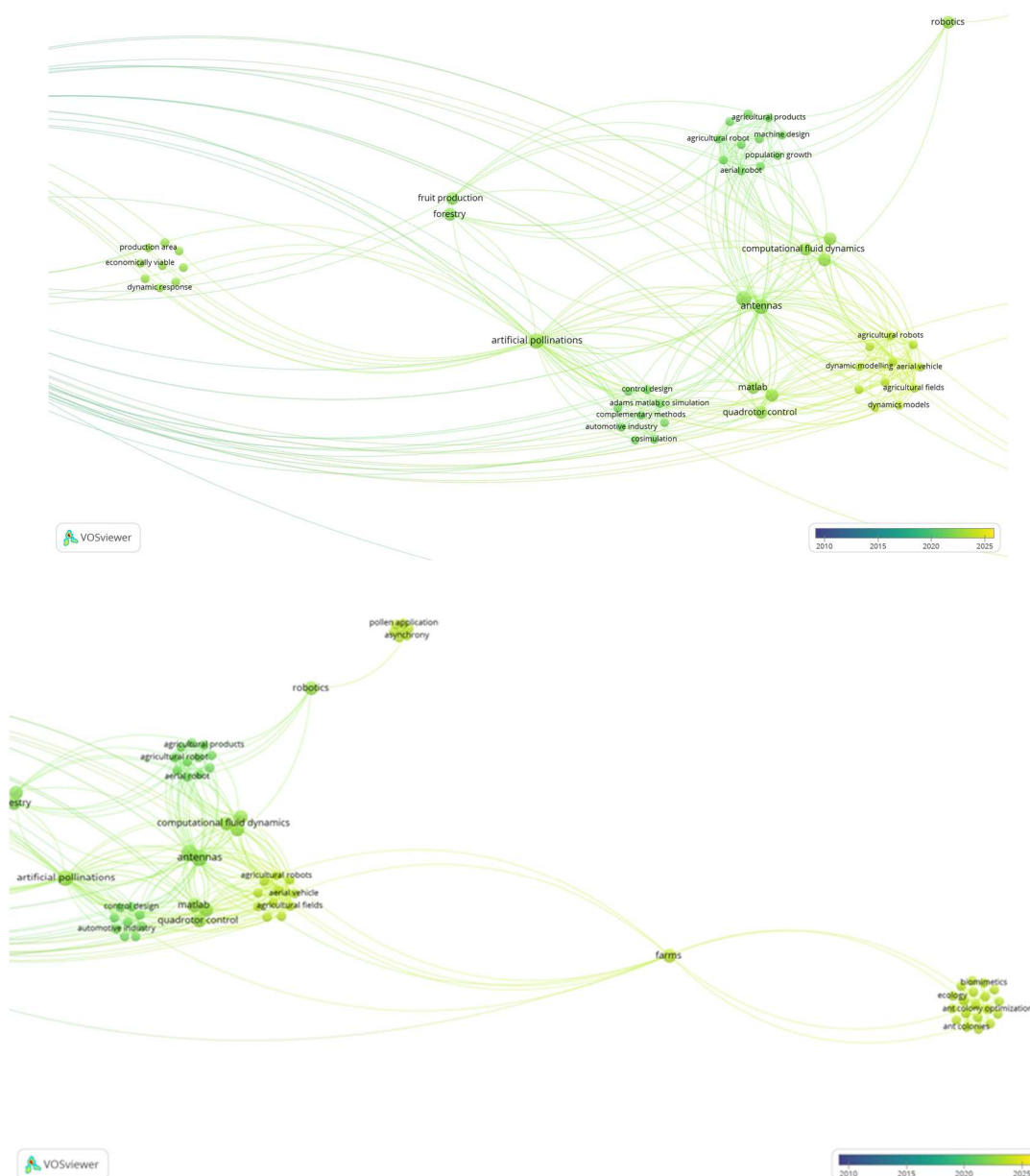


Figure 3. Co-keyword network visualization based on “Artificial” and “Pollination and Technologies and Walnut”. Results were based on Cluster 1 which is defined by 52 keywords (items).

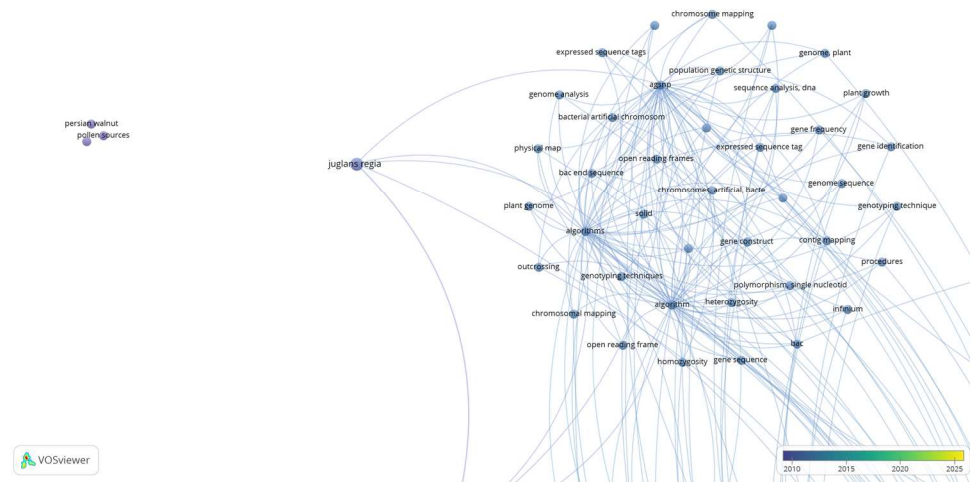


Figure 4. Co-keyword network visualization based on “Artificial” and “Pollination and Technologies and Walnut”. Results were based on Cluster 2 which is defined by 45 keywords (items).

Based on simulation methodologies for controlling a quadrotor, MATLAB provides a solution of standard flower pollination algorithms with more information on Nature Optimization Algorithms provided in a book entitled “Nature-Inspired Optimization Algorithms”. In detail, the flower pollination algorithm (FPA) is a population-based metaheuristic optimization algorithm derived from the evolution of flowering plants [40]. Following its original development, FPA was modified and hybridized to improve its performance for different optimization problems such as the bee-pollinator concept to solve the data clustering problem [41]. A modified version of the FPA, which utilizes the crossover techniques for resolving multidimensional knapsack problems, was also developed [43]. Keeping the above-mentioned facts in view, we believe that an algorithm such as the FPA, should be used for a pollination robot to prevent or reduce the risk of the walnut blight disease.

In addition to the FPA, the bacterial microbiota associated with flower pollen is influenced by the pollination type and exhibits a significant degree of diversity and species-specificity [42]. So, for microbes–pollen interaction presumably we must know the sequencing of 16S rRNA gene amplicon libraries to identify dominant microbe (-s) phylum and core microbiome of pollen from different walnut cultivars. Since pollen-associated bacteria may have a potential impact on walnut blight disease pollen microbial communities from different walnut cultivars are necessary or need to identify. That information could play a significant role in the flower pollination algorithm and develop a strategy that leads to new valuable information on walnut cultivars microbes–pollen interaction during pollination.

Moreover, considering the algorithm-based approach, is necessary to provide criteria for the aerial robot path. Basic simulation steps helped determine pollen streams under the quadrotor, unmanned aerial vehicle [28]. Those steps are the following:

1. Design and fabrication of quadrotor components

For the quadrotor to be able to carry and distribute the pollen over trees, the quadrotor components must be selected correctly i.e., the body size was chosen based on the pollen tank. The objective of the quadrotor was to pollinate, so the tank was equipped with a nuzzle with four holes to allow pollen to fall uniformly. It is recommended that the propulsion system uses a motor with a lower current. The electronic devices contained various sensors, such as a compass, a global positioning system (GPS), a barometer, and connections for the flight control system [28].

- ## 2. Modelling and Control

The airflow under the UAV was simulated using computational fluid dynamic (CFD) software. This simulation helped determine pollen streams under the robot to ensure that the released pollen reached the target trees. The quadrotor controller was designed and simulated in MATLAB, for system stability [28].

3. Pollination of walnut trees

Different mixtures of pollen diluted with talcum powder were distributed over different groups of trees, using the quadrotor. After a few months, the results achieved on the trees were measured and the merits of the proposed system were evaluated [28].

5. Machine Learning and Pollination

Machine learning (ML) can play a significant role in the context of pollination, offering innovative solutions and insights to address challenges and optimize pollination processes. Below several ways in which machine learning is applied to pollination are mentioned:

1. **Predictive modeling:** ML algorithms can analyze historical data on pollination success, environmental conditions, crop yields, and phenology to create predictive models. These models can help predict optimal times for pollination, considering factors such as weather patterns, bloom asynchrony, and the availability of pollinators [43–45].
2. **Automated monitoring:** ML-powered monitoring systems can analyze images or sensor data to track the health and development of crops. This real-time monitoring allows for the early detection of issues related to pollination, such as low pollination rates or the presence of pests that could affect pollinators [46,47].
3. **Optimizing pollination strategies:** Machine learning algorithms can optimize artificial pollination strategies based on a variety of factors [48,49]. These include the type of crop, environmental conditions, and the efficiency of different pollination methods [50,51]. This can lead to more targeted and effective pollination efforts [52].
4. **Identification of pollinator behavior:** ML can be used to analyze the behavior of natural pollinators, such as bees, by processing video footage or sensor data. Understanding pollinator behavior can provide insights into their preferences, movement patterns, and efficiency in pollinating specific crops [53,54].
5. **Genetic analysis:** Machine learning techniques can analyze genetic data related to plant characteristics, including traits associated with pollination [55,56]. This information can contribute to breeding programs aimed at developing crops that are more resilient to environmental challenges and more compatible with artificial pollination methods [57,58].
6. **Decision support systems:** ML-based decision support systems can assist farmers in making informed choices related to pollination strategies. These systems can consider a range of variables and provide recommendations for optimal pollination practices [59,60].
7. **Data integration:** Machine learning excels at integrating and analyzing large datasets from various sources. In the context of pollination, this can include data on weather conditions, soil quality, plant health, and more. The integrated data can provide a comprehensive understanding of the factors influencing pollination success [61–63].

While machine learning holds great promise in improving pollination processes, it's essential to recognize the importance of interdisciplinary collaboration between machine learning and pollination as presented with VOS viewer analysis in Figure 5. Based on Figure 5, up today knowledge, 2022, (Figure 5 items with yellow dots) requires: "anatomy and histology", "classification of information", "classification algorithms", "image classification", "neural networks", "population statistics", "robotics", "machine learning", "deep learning" and "meta-heuristic algorithms" (Figure 5, items with yellow dots).

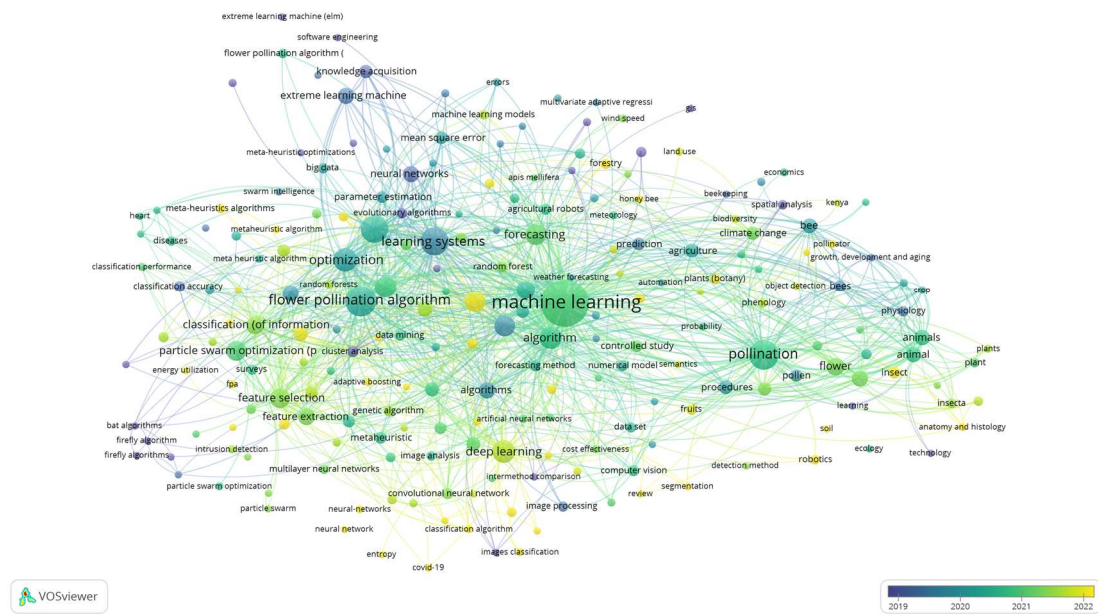


Figure 5. Co-keyword network visualization based on “Machine Learning” and “Pollination”.

Even more, using the VOS viewer mapping software and the Scopus bibliographic database based on the search strategy criteria “Aerial and Pollination” (Figure 6), all the above-mentioned criteria or technologies show promise but they still require important improvements such as “antennas”, “aircraft detection”, “aircraft control”, “MATLAB”, “internet of things”, “population diversity”, “flower population algorithm”, “swarm intelligence algorithm”, “learning algorithm”, “learning systems”, “deep learning”, “quadrotor control”, “image enhancement”, “auxiliary pollination”, “supplementary pollination” and “convolutional neural networks”, Figure 6 (items with yellow dots).

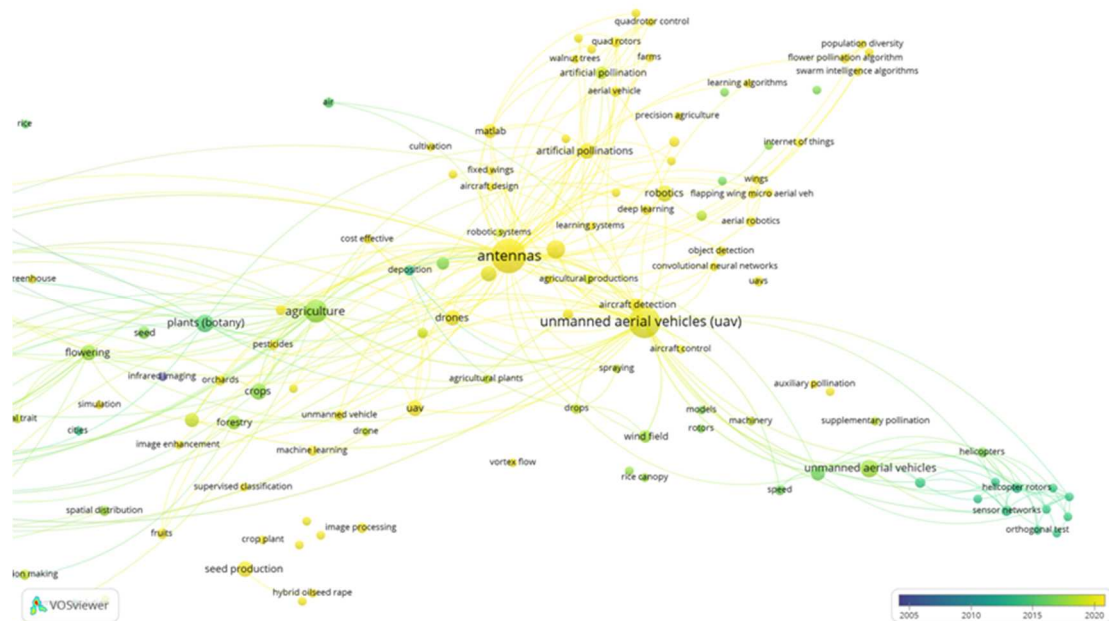


Figure 6. Co-keyword network visualization based on “Aerial” and “Pollination”.

6. Walnut Pollination. Model to prevent or reduce the risk of the walnut blight disease (our view).

Developing a walnut pollination model involves creating and a predictive framework that considers various factors influencing the pollination of walnut trees [29,41]. Keeping in mind the success of the walnut pollination model (Figures 1 - 6) and based on the above paragraph we illustrate the following flowchart (6 steps, state of the art flowchart), which provides a general guideline for conducting a state-of-the-art analysis for a pollination robot to prevent or reduce the risk of the walnut blight disease.

State of the Art Flowchart

- 1st Step. Walnuts: budbreak, bloom, and pollination growth stages
 - 1.1. Identify spring bud break, leaf emergence and anthesis growth stages of the walnut cultivars.
 - 1.2. Understanding how plants grow and develop during bud break-up, anthesis, and pollination.
 - 1.3. Understanding protandrous, protogynous mechanisms prior to the pollen shedding.
- 2nd Step. Study the bacterial microbiotas associated with the pollen
 - 2.1. Identify the “core” pollen microbiota.
 - 2.2. Compare bacterial abundance and diversity between walnut cultivars.
 - 2.3. Assess the impact of the pollination type on the variability of the pollen microbiotas.
 - 2.4. Estimate the role of *X. arboricola* pv. *juglandis* to stigma by contaminated pollen.
- 3rd Step. State of Walnut Pollination – Develop Pollination Algorithms
 - 3.1. Check reservoir cultivars i.e. Chandler of inoculum presence.
 - 3.2. Check for conditions that encourage the disease to spread such as moisture, and especially the combined action of wind and rain.
 - 3.3. Check for air-borne inoculum when catkins open.
 - 3.4. Check for pistil- late flowers.
 - 3.5. Collect and store uninfected pollen.
- 4th Step. State of Walnut Pollination – Develop Pollination Algorithms
 - 4.1. Set genetic algorithm for ‘mutation’, ‘selection’, ‘recombination’ based to pollen – microbes’ interaction.
 - 4.2. Use metaheuristic algorithms which included differential evolution
 - 4.3. Use the appropriate flower pollination algorithm which is inspired by the pollination process.
- 5th Step. Design of an Autonomous Precision Pollination Robot
 - 5.1. Quadrotor (pollinating drone) to carry pollen grains.
 - 5.2. Pollinating drone to make an ideal delivery system, landing on the pistil of a flower to result in pollination.
 - 5.3. Drone technique would need some refinement in localization and mapping and control.
 - 5.4. Metaheuristic optimization algorithm to find the best (feasible) solution out of all possible solutions of pollination optimization problem.
- 6th Step. Algorithm Optimization
 - 6.1. Analyze historical data on pollination success, environmental conditions, and crop yields (machine learning)
 - 6.2. Use flower pollination algorithm parameters tuning (formulating the above steps mainly step 1 and 2 due to climate change).
 - 6.3. Start the process from step 3 and improve equipment at step 4 and 5.

7. Conclusions

Evidence to date is convincing that microbes such as the bacterium *X. arboricola* pv. *juglandis*, occur in pollen and influence walnut blight disease. Research showed that *X. arboricola* pv. *juglandis* the causing agent of walnut blight can affect the leaves, stems and nuts of the tree even pollen. In this review we present a flow diagram of a new artificial pollinator that we assume can reduce the spread of the disease in the orchard. Considering the knowledge of a solution to overcome this bacterial plant infection, VOS viewer suggests that the foundation for a new, more sophisticated and efficient walnut pollination model to prevent or reduce the risk of the walnut blight disease is possible. So,

the purpose of the ideas presented here was to facilitate a description of such a pollination model with fundamental components, the identification of the 'core' pollen microbiota, specify an appropriate flower pollination algorithm, design an autonomous precision pollination robot and minimize the average errors of flower pollination algorithm parameters through machine learning and meta-heuristic algorithms.

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