Adaptation to climate change of blackcurrant variety ”Noir de Bourgogne” through plant breeding and pollination optimization

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Figures and tables

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1) Introduction

1.1 Climate change and agriculture

Climate change (CC) is accelerating and intensifying, and the human influence on this process is now unequivocal (IPCC 2021). CC is coming with a biodiversity collapse. At global scale, IPBES (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services) estimates a decline since 1970 of 40% of terrestrial species (75% of them are insects). Around 10% of insect species are threatened with extinction. (Bridgewater et al. 2019). This loss of species diversity is followed by a decline in population sizes as the Global Living Planet Index suggest. It estimates an average decline of 68% in the size of populations of mammals, birds, amphibians, reptiles and fish tracked between 1970 and 2016 (Almond, Grooten 2020). Agriculture have an important role in these alarming observations: agriculture and climate change are highly interconnected.

Impacts of agricultural production on CC are numerous: 80% of global forest deforestation (FAO 2016), 70% of biodiversity loss (Almond, Grooten 2020), 24% of total global anthropogenic emissions of greenhouse gases (Smith et al. 2014 ; Tubiello et al. 2014) are due to agriculture intensification. This sector accounts for 70% of global water consumption and it is the single-largest contributor of water pollution (FAO, CGIAR 2018 ; UN 2018). However, agriculture is also facing increasing threats from weather events due to climate change. In temperate cropping regions, drought and heat affects the crop lifecycle at critical developmental timepoints (Snowdon et al. 2021). Anthropogenic Climate Change has reduced global agricultural total factor productivity by 21% since 1961 (Ortiz-Bobea et al. 2021). CC is predicted to have negative impacts on food production, food quality and food security in the future (Atkinson et al. 2008). For example, crop productivity is expected to decrease from 6 to 19% if temperatures increase of 2 °C in the future (Zhao et al. 2017). In addition to weather events, crop production is frequently limited by a lack of pollinators (Reilly et al. 2020). Animal vector pollination is an essential ecosystem service for agriculture: around 90% of cultivated plants (angiosperms) benefit from animal pollination (FAO 2018). Pollinators, such as bees, birds and bats, contribute to 35% of global crop production, increasing the production of major food crops around the world by 75 % (Klein et al. 2007). However, due to the decline in global biodiversity, this ecosystem service is threatened. Pollination is one of the "regulatory services" provided by ecosystems, and especially by insects. Since 1990 in Europe, insect populations have fallen by almost 80% (Sánchez-Bayo, Wyckhuys 2019).

To limit these effects, adapting crops to climate change has become an urgent challenge, which requires some knowledge on how crops respond to those changes (Ceccarelli et al. 2010). Plant breeding can be an appropriate tool in this purpose with complementary management changes. In the past, most of plant breeding works were based on one objective (resistance to a pathogen or pest) (Chapman et al. 2012). However, in this 'climate change' era, the challenge is to breed new varieties for improved resistance to a diversity of abiotic and biotic stresses at the same time (Badjakov et al. 2017). This context is difficult for all types of crops, but can be even more problematic for the less widely cultivated crops, such as blackcurrant.

1.2 The specificities of blackcurrant crop

Blackcurrant (Grossulariaceae, Ribes nigrum L.) is a bushy fruit shrub. It is produced in different parts of the world (EU Canada, New Zealand, Russia…). In EU, Poland produces the main part: 130 000t/year of the 170 000t/year in EU (CERD). France is the third producer in Europe with 7000 t/year (Interfel, 2019). The French production is oriented towards the quality of the product (Froissart, Prunier 2019). This is particularly the case with the production of the Noir de Bourgogne variety (NB) in Burgundy. The NB have a very particular aroma, quite distinct from other varieties (Jung et al. 2017). This aroma is highly valuable for the production of the liquor "Crème de Cassis". A rate of NB variety is required to produce the « Crème de cassis de Dijon » and « Crème de cassis de Bourgogne», two products protected by an IGP (Indication Géographique Protégée) (INAO 2014). There are 880 hectares of blackcurrant crop in Burgundy. Among them, the most represented variety is the NB one. (75% of total production in Burgundy) followed by Royal de Naples (RN) and Blackdown (Froissart, Prunier 2019).

This NB variety got poor yields in recent years (less than 3t/ha), lower or equal to the profitability thresholds expected by farmers (Baillard, 2020). These low yields can be explained by various factors. The first one is abiotic with heat and frost at key growth periods. The second factor is biotic stresses by significant pressure from crop pests and a lack of blackcurrant pollinators (Baillard, 2020). Blackcurrant have a mixed pollination system based on both self and cross-pollination. This lack of blackcurrant pollinators affect more or less some blackcurrant varieties depending on their main pollination system. NB variety is known to be a predominantly allogamous variety. It requires allopollination by another variety in orchards, most often the Royal de Naples variety as pollen donor. In a row, one Royal de Naples bush is generally planted every five NB plants.

The lack of genetic biodiversity can also explained the NB yield. The domestication of blackcurrant is recent (Anstett, Treveret, et Louâpre 2019). The production of blackcurrant for its fruits have been developed in the middle of the 19th century, following the invention and distribution of crème de cassis by Denis Lagoutte (Guenin 2003). The local varieties produced by farmers before the 1980s were open-pollinated varieties. They have been mostly lost over time. Nowadays, three clones are present in crops, coming from the last plant breeding process done in the 1980s. There are three cultivars heterozygotes obtained by INRA selection. (Petermann 2020). These clones are propagated and planted as cuttings. Wild blackcurrant is still present on site but has weak interests, particularly organoleptic ones. Therefore, the genetic diversity of blackcurrant crop is limited.

1.3 “Programme d’Innovation Européen”

To find suitable solutions, blackcurrant producers in Burgundy have created an inter-branch grouping (“association loi 1901”) including blackcurrant producers, agricultural cooperatives and liquor makers under the name "Les acteurs du cassis". This group has associated with technical and research collaborates (SAYENS, CNRS, Agrosup Dijon, Spiral…) to implement agroecological actions for the sector’s sustainability. Together, they developed a “Programme Européen pour l’Innovation” (PEI) in 2018, a four-year program in collaboration with the Bourgogne-Franche-Comté region and EU (FEADER). An application have been submitted this summer 2021 to continue for next two years. This collaborative project was built around several research topics: i) the selection of traits for plant breeding and fight against a blackcurrant pest, the white peach scale (WPS) *Pseudaulacaspis pentagona, ii)* the optimization of pollination, iii) plant sanitation, finally iv) an organoleptic component (Réseau Rural Français 2020). Because of unexpected results provided by the partners since the beginning of the project, some project goals were refocused to new actions.

Selection of traits for plant breeding

Plant breeding (first topic) was initially focused on increased yield by obtaining F1 with productive varieties and backcrossing with Noir de Bourgogne. It was important to preserve the NB for its organoleptic qualities, but it was also essential to implement new traits coming from other varietal background, and increase genetic diversity. The first traits were selected on phenology and abortion rate criteria. As plant breeding program is a long-term process, two other traits have been added over time to save time, self-fertility and resistance traits.

The phenological trait was fruiting precocity (to avoid heat wave and summer drought, which can have a negative impact on fruits). Flowering lateness was also a wanted characteristic (to avoid spring frosts, when flowers are already developed). Phenology observations were carried out over two years on a varietal collection (2019-2020). Also, NB blackcurrant undergoes an important abortion rate: many flowers and fruits fall before the harvest period. At the beginning of the PEI project, the cause of this abortion phenomena was unknown. It was necessary to learn if the abortion rate is variety-depending. In this case, it will be essential to integrate “the abortion rate trait” in the plant breeding program, to select varieties with a low abortion rate.

The third trait concerned self-fertility. Some varieties like NB rely more on outcrossing and presence of pollinators that highly decrease yield in the current collapse of insect’s population. Thus obtaining a variety less dependent on pollinators would be useful to secure future blackcurrant yield. NB and RN were said to be self-sterile (Doré, Varoquaux 2006), but recent work show that it is in fact partially self-compatible (Duchet, Anstett, com.pers.). To measure self-fertility, Anther Stigma Distance (ASD) is a classical proxy of ability for self pollination (Jia, Tan 2012) ASD is defined as the shortest distance between the top of the anther and top of the style of the same flower. ASD is closely linked with herkogamy (the spatial separation of male and female structures in flowers). Two subtypes of herkogamy were considered in this study: approach herkogamy (anthers below the stigma, with a short filament), and reverse herkogamy (anthers above the stigma, with a long filament) (ANON. 2019). The possible self-fertility (through a long filament trait) was defined as a good indicator to select the valuable varieties for plant breeding.

To test whether ASD could be a proxy of selfing rate in blackcurrant crop, first observations and measurements were done in 2019 (Bouidghaghen 2019). ASD measurements had been performed in 2020 on twelve parental varieties of a varietal collection (Denis et al. 2020) suggesting important variation between varieties. Results have shown that varieties Andega, PC 110, 88-04-181, and Ben Tirran (blue, Figure. 1) had presented anthers above the style (ASD>0, long filament), Tiben flowers have anthers and style at the same level, while the others varieties (red, Figure 1) have flowers with anthers lower than stigma (ASD<0, short filament trait) (Denis et al. 2020). These ASD measurements were compared with yield per variety: low yield varieties tending to have negative ASD. However, the direct correlation between selfing rate and ASD is still to be documented.

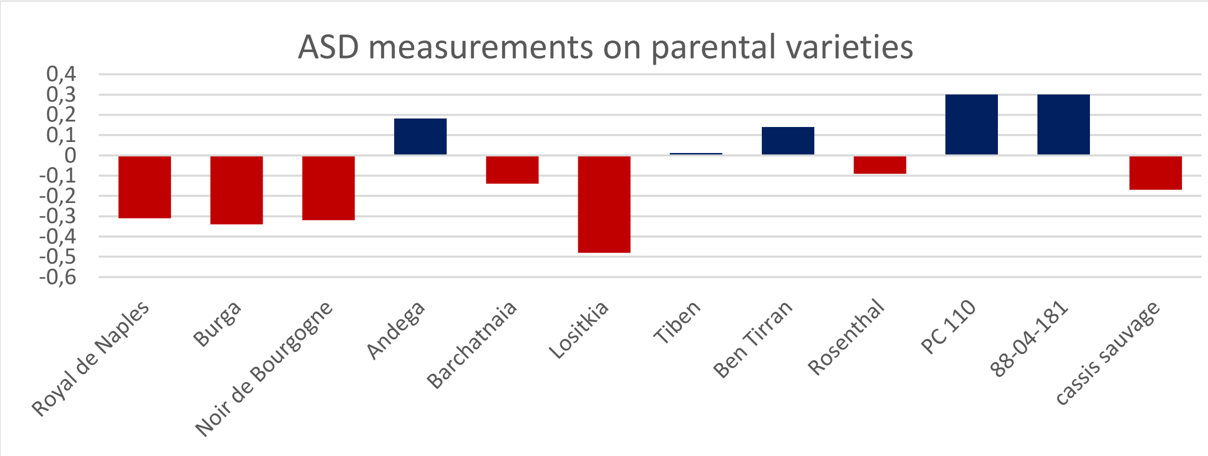

Figure 1 Average anther stigma distance for the varieties used on parental varieeties (Marine CHASSERAY-NARS, SAYENS SATT, 2020 (Denis et al.2020)

The fourth trait integrated in the plant breeding process was the resistance to White Peach Scale (WPS). This hemiptera *Pseudaulacaspis pentagona* develop very quickly and can destroy blackcurrant orchards which are uprooted. In France, WPS is one of the main threats to blackcurrant crop especially in Burgundy, the Loire valley and the Rhône-Alpes regions (Kuzmin et al. 2020). It has been treated for years with chemicals based on Pyriproxyfen (example of ADMIRAL PRO, PHILAGRO). Several insecticides from this same chemical family have been gradually withdrawn from the market because of their harmfulness (E-PHY ANSES). Currently, there is no appropriate phytosanitary solution to manage this pest in a durable way. It was essential to include in this PEI program a sustainable management of this pest, whether through varietal resistance in the plant breeding program or the search for suitable auxiliaries and parasitoids. In the field, colonization by WPS is quite unpredictable and patchy. Even if in highly infested orchards, a higher sensitivity of NB comparing to RN is easy to notice, measurement of varietal resistance to WPS is difficult to perform. In the varietal collection, three individuals of each genotype are grown side by side. Uninfected plants may be either resistant plants or only not yet infested. In order to develop a practical test of blackcurrant resistance to WPS, we achieved several assays of artificial infestation of blackcurrant cuttings with WPS originating from lab rearing.

The WPS lab rearing follow the same lifecycle than in natural conditions. WPS females spend the majority of the cycle immobile because they are attached to the host plant for food. They are well protected under a shield for almost all their life. This shield is created by incorporating the molded skins of her previous molts. Females begin to lay eggs about two weeks after mating and will continue for ten more days ([1], Figure 2). Females can laid a maximum of two hundred eggs under their shield (Duchet, pers comm.). Once laying is complete, WPS female dies a few days or weeks later and eggs hatch ([2], Figure 2) and come out of the shield. Young crawlers move on another area of the host plant ([3], swarming, Figure 2) before settling down. The fixation of crawlers begins with inserting the stylet into the host plant for food, and then continues with the fabrication of a shield for females or a cocoon for males ([4], Figure 2). It is at the end of fixation that the morphological differentiation between the two sexes occurs and the adult stage start. Males emerge from their cocoons ([5], Figure 2), as colored adult with wings in order to be mobile for mating ([6], Figure 2). Male live for a short period (approximately 24 hours) (Branscome 2019). The main difference between WPS lab rearing and in natural conditions is the time required for the development of each generation. In lab, a generation can be completed in two months, while WPS makes two generations in Burgundy. In 2020, first crawlers appeared from May 15 for the 1st generation, and July 28 or 29 for the second generation in blackcurrant orchards of Côte d'Or (Cyrielle Denis, pers. comm,).

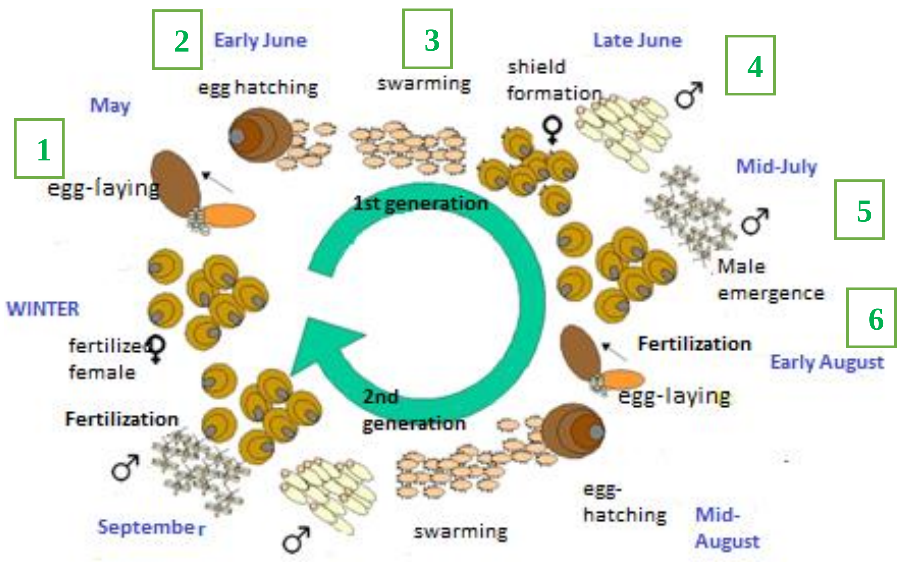

Figure 2 Biological WPS lifecycle (Trillot et al. 2001)

WPS infestation on blackcurrant is localized towards the base of the plant, inside the bush. Female can be developed on the bark or may be located in a crack in the bark of the wood. WPS are recognizable in the field by white spots on the wood (shields) and white residues (cocoons) (Harzer et al. 2018). The main difficulty to control this pest in the field is due to the duration of the "sensitive time" of WPS. They are sensitive to insecticide only during the crawlers swarming when WPS females are not yet protected. This swarming stage usually last twelve hours, and crawlers are very mobile. This is also problematic for the study as it can be difficult to obtain eggs at the right stage of development to develop a WPS population in lab.

Optimization of pollination

The second research topic studied in the PEI was concerning blackcurrant pollination. The causes of the NB yield loss was not precisely known at the start of the PEI project. A first response was given when the link between yield loss and the importance of pollination has been made during the project. The second topic of the PEI 2018 program was focused on the recovery of the pollination ecosystem service in blackcurrant orchards.

Bumblebees and wild bees were often cited in the literature as the main pollinators of blackcurrant (Grégoire 1984 ; Fountain, Hopson 2013 ; Fliszkiewicz et al. 2011). A data survey on the abundance of NB pollinators in Côte-d´Or in recent and ancient literature has been carried out in 2018. Since 1979, the number of bumblebees (*Bombus)*, *Apis* and of *Andrena* were divided by a factor close to 100. A majority of *Bombus*, and a few *Andrena* and *Apis* were the only species observed in the field. *Bombus* were more numerous in the middle of the plot and less abundant around plots, showing the attractiveness of blackcurrant fields (Anstett et al. 2019). After these observations, insect hotels were left in 2020 in farmers' fields during the entire season to identify local pollinators which remained on the blackcurrant plot. The main pollinating insects found were identified belonging to the genus *Osmia*. It is a genus of hymenopteran insects, a solitary mason bee, which has a much smaller flight distance than bumblebee dispersion distance. Another experiment was carried out to measure the pollination impact on fruit formation and final yield on *Bombus* pollinators. The purpose was to compare 'Noir de Bourgogne' yields in "natural pollination" and in "optimal pollination" contexts. With bumblebee hives, twice as much flowers present at the start of the season had formed a fruit compared to natural pollination. "Optimal" pollination had allowed multiplying the yield by 3.56 on average, depending on the plot and its landscape context. The aim in 2021 was therefore to continue this experiment to measure the impact of pollination by adding osmia shelters and bumblebee hives in field conditions (pollinators could move freely in and out of the plot.) (Denis et al. 2020).

1.4 Experiments of the internship

The PEI program allows a strong interaction between partners of the project. This internship was co-supervised between SAYENS (focus on plant breeding program) and CNRS-Biogéosciences (focus on pollination). The main point of this internship was to improve the collaboration with these two partners. Three experiments were followed during this period: 1) phenology/self-fertilization, 2) measurement of blackcurrant resistance to WPS (both in the plant breeding program) and 3) impact of adding *Osmia* shelters and *Bombus* hives on blackcurrant yield (pollination).

The purpose of this internship for the plant breeding program was to save time to compare and select the most appropriate traits for selection. The aim of the first experiment (phenology/self-fertilization) was to study varieties available for crossing, in order to have a better knowledge on their lifecycle linked with weather events, and their degree of herkogamy. First, it was necessary to figure out which varieties are the most adapted to resist to each type of climate events. To answer this question, a followed-up of phenological stages on a varietal collection was carried out to determine early and late varieties over years. Moreover, an emphasis was done on eleven parental varieties of this collection to estimate their fruit initiation. As ASD measures were carried out last year on these varieties, we studied the correlation between ASD and fruit initiation. This relation can determine if ASD is a good indicator of self-fertilization for blackcurrant flowers. Also, if the two variables are correlated, it can help us to save time to select varieties to cross. ASD measures, initially done on parental varieties, were repeated this year on hybrid groups. These measures could provide to better understanding of the phenotype of long filament trait in hybrid F1 groups. Depending on the representation observed of this ASD variable in hybrid groups, it could be possible to determine which individuals should be selected for plant breeding.

The second experiment (blackcurrant resistance to WPS) was to develop a resistance test on blackcurrant cuttings. Several cuttings of various varieties were infested with WPS eggs. Varieties have been chosen depending on their resistance to WPS observed on the varietal collection over years. As WPS infestation is not common in the literature, the main purpose of this experiment was to identify the main obstacles to its implementation. In addition, if infestations worked, a comparison between resistant or sensitive varieties can be achieved to analyze varietal resistance to this pest.

The third experiment was focus on the restoration of the pollination service in the blackcurrant orchards. The project consisted in the installation of *Bombus terrestris* hives or *Osmia* shelters (two species were studied: red mason bee *O. bicornis* or horned osmias *O.cornuta).* The aim was to measure the pollination impact on the fruit initiation rate and fruit production of Noir de Bourgogne in blackcurrant orchards.

2) Material and method

2.1 Plant material

Climate of the Côte-d'Or is oceanic with a semi-continental tendency. The two main experimental sites were located in Bretenière and Merceuil, Côte d'Or. The entire varietal collection of *Ribes sp*. was relocated in 2015 to Bretenière, Côte d'Or, France (47°14'00.3"N 5°06'35.7"E) with both varieties for fruit production (especially for liquor) and varieties for bud production (for perfumery). The study was focus only on fruit blackcurrant varieties, which represented one hundred varieties in total in the conservatory. All the varieties are presented in the appendices Each accession is represented by three plants. They come from countries of the four main blackcurrant-growing regions in the world: Western Europe, Eastern Europe, Scandinavia (Sweden), and North America (Canada). Forty-five accessions are old varieties (no longer protected by a COV (Certificat d'Obtention Végétale). Twenty-three varieties come from a breeding program of INRA Angers. Seven varieties are licensed for experimentation. Moreover, the Côte d'Or Chamber of Agriculture had introduced nine varieties present in farmers' fields. Five new cultivars (James Hutton Institut) had been implemented in 2020. Plants are irrigated and soil is covered by a tarp.

In 2018, crosses were initiated between 10 parental varieties chosen and NB as pollen donor. All the parental varieties have been selected for their resistance to WPS and for their resistance or tolerance to powdery mildew. These parental varieties presented a large genetic diversity, they are all quite different genetically from NB and between them. (A phenogram of the varietal collection is presented in the appendices Seeds obtained from crosses were germinated to obtain five hundred fifty F1 individuals. These hybrid F1 were planted in several places: hybrid from seven crosses at Bretenière, hybrid from ten crosses at Merceuil, hybrid from two crosses directly in a farmer's field at La Buissière-sur-Ouche, and hybrid from two crosses at Agrosup Dijon plateform. F1 hybrids studied here were all genetically different. (compared to a crossing from inbred lines). F1 plants presented at Agrosup and Bretenière are potted, the others are grown in the open ground.

2.2 Collection and hybrid populations follow-up

2. 2.1 Phenology

Weekly phenology data were taken during the flowering season (April-May) and twice a month at other stages. These observations were performed on the hundred varieties of the collection and the seventeen hybrid populations in Bretenière and Merceuil sites. Phenological stages are described in a document of Chambre d'Agriculture Côte d'Or, the international BBCH system adapted to blackcurrant culture (Appendix I)

2. 2.2 Flowering and fruiting phenology

Flowering and fruiting phenology was recorded weekly on 14 varieties of the Bretenière collection. For each variety three branches were marked on three individuals on April 27 (total of 126 branches). In Merceuil we followed only one branch on ten different F1 individuals from Andega variety, chosen for their important flowering. Since floral phenology varies with the location of the flower, we focus floral phenology of the extremity (20cm) of each weekly.

The date of several key flowering stages have been recorded (Figure 3): date of mid-flowering (F2 stage of development: 50% flowering); date of beginning of fruit development (I3: full fruit initiation), end of fruit development (8.1/8.7: ripeness)

F2 /65 - 50% de fleurs ouvertes | I3 /79 - Nouaison complète | 87 - Maturité complète

Figure 3 Development stage studied for the flowering follow-up.

To estimate abortion rate, at F2 stage, the total number of flowers on the extremity of each branch i.e. the sum of open and closed flowers plus the number of pedicels scars were counted. At full fruit initiation (I3) and ripeness stages, the number of fruits were counted. Fruits harvested have been counted and weighted per variety. The fourteen varieties used for the flowering follow-up in the collection were harvested. Harvesting was executed manually on the three plants. The weight of one hundred berries and the total weight were measured for each variety. The hybrid populations cannot be compared to bushes planted in the open ground: they were too young to be harvested; growth is different depending on the cropping method).

2.3 Measurement method of Anther Stigma Distance

To document variation in ASD for hybrids, about twenty freshly opened flowers were harvested in April and May during the F2 stage (50% flowering). It was done on 450 individuals of seven hybrid groups located at Bretenière and Merceuil sites. The samples were kept in alcohol at 80%. A flower sample was dissected under a stereomicroscope. (The floral formula of blackcurrant is (5S) + 5P + 5E + (2C) (Bouidghaghen 2019), there had to be three stamens and the style on one side, and two other stamens on the other side). The sample was placed on a support made of paraffin balls. ASD measure was done by subtracting anther and style height, from a line traced above ovary (Figure 4). The measurements were taken with a binocular magnifier Axiocam 208 ZEISS with the software ZEN (Objectif: 1x, Zoom: 0.8x, Reflector: BF, Adaptater: 0.5x, Total magnification: 0.4x).

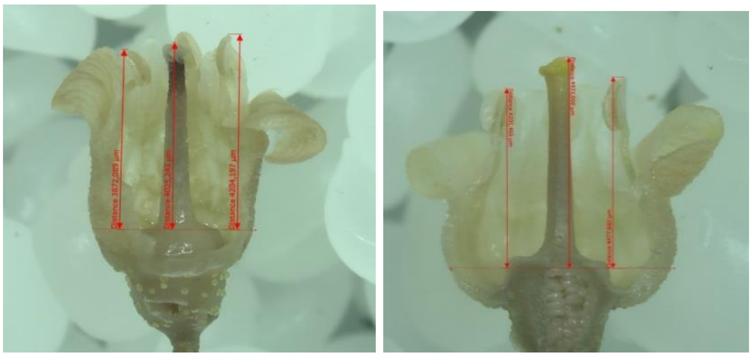

Figure 4 Measurement of anther and style height to estimate ASD.
(Left: one anther is above the stigma, Right: both anther are below the stigma)

These measures were repeated on ten flowers per individual for the first hybrid F1 cross, then reduced to six flowers for the next crosses after a statistical power test. The hybrid collection was too numerous to perform all the floral ASD measurements during the internship. The following hybrid F1 were studied: NB * Andega (37 individuals), NB * Lositkia (12 individuals) and NB * Ben Tirran (20 individuals). The parental varieties of NB * Andega and NB * Ben Tirran crosses presented short filament (from NB) and long filament traits (Andega and Ben Tirran). NB * Lositkia have short filament trait from the two parental varieties, according to ASD measurements done in 2020 (Denis et al. 2020).

2.4 Development of WPS infestation test

2. 4.1 Material

Several methods have been tested to develop a repeatable measurement of blackcurrant resistance to WPS on young cuttings.

Plant material

One hundred sixty cuttings of nineteen varieties were taken from the Bretenière varietal collection in January 2021. Each variety had eight or twelve replicates. The cuttings were repotted and placed in a culture chamber at the Université de Bougogne, Dijon in March 2021. A temperature logger was placed in the culture chamber to monitor room temperature. Room conditions were about 24°C and 50% of humidity. The room was lighted with a horticultural lamp, on a cycle of 18 hours day, 6 hours night.

Animal material

A rearing of WPS at the Université de Bourgogne have been developed since 2019 from WPS eggs sampled in a field. The breeding was developed on potatoes. Potatoes infested were placed in boxes protected by tulle fabric. A weekly or bi-monthly follow-up was carried out to know the WPS development stages for each box, and to be able to renew the breeding with new potatoes. The room conditions were similar to the plant material.

2.4.2 Method

Development of infestation method

When a sufficient number of female WPS were laying eggs, two hundred eggs were collected then deposited on each cutting. To ensure that eggs hold in place close to the bark we tested several ways to secure it. We tested i) a cone made from a post it note, but this technique crushed some of the eggs deposited when the cone was removed, ,ii) a string but some of the eggs were not in direct contact with the cutting, iii) a piece of paper towel secured by a freezer bag link. This method was convenient for egg development but quite difficult to install properly (the tightening of the paper towel was different from one cutting to another) and iv) a cone made of cigarette paper. We retained this last method because it was the most repeatable method. The tightening of the cigarette paper was identical from one repetition to another due to the glue present on the paper. Moreover, since cigarette paper is very thin, the step of removing the cone (once the infestation is over) was made easier with this method. First, this test was performed on two cuttings of Troll variety with paper towel. Then, the two methods have been tested on NB, Leandra, Ontario Climax. Finally, paper towel method have been set aside, RN have been infested only with a cigarette paper cone (Table 1).

Table 1 Cutting varieties and its resistance to WPS per infestation method

Variety	Putative resistance	Infestation method	
		paper towel	cigarette paper
Troll	susceptible	2	0
NB	susceptible	1	1
Leandra	resistant to WPS (negative control)	1	1
Ontario Climax	susceptible (positive control)	1	2
Royal de Naples	susceptible	0	2

Varietal resistance test

Since egg availability strongly reduced the number of tests we could perform, only eleven cuttings were tested, nine from sensitive varieties and two for putatively resistant varieties. Two varieties were used as controls: Leandra, a variety known for its resistance to WPS and Ontario Climax, a variety, on the contrary known for its susceptibility. (This information regarding resistance had been observed in previous years on the varietal collection at Breteniѐre platform). The NB and RN varieties had also been chosen, because they are the most widespread in blackcurrant orchards. According the literature, both varieties showed some sensitivity to WPS. The infestation was repeated on at least two cuttings per variety (Table 1). This low number of repetitions allowed descriptive statistics to visualize data and to have a first approach.

Monitoring and measurements on infested cuttings

After depositing WPS eggs in the cone, the cutting was placed in an emergence cage. Since only males of WPS can fly, several cuttings could be placed in a single cage only if they were not in contact with each other. Due to the management of the WPS breeding and the difficulty of having enough eggs available on the same date, infestations were executed on different dates. Approximatively forty-five days were necessary to have a WPS fixation on the cutting. After this period, the cone was removed and the infestation point was marked with a pin pressed into the bark. The first WPS generation were observed at this date (infestation + 45d). The counting was performed with the same binocular magnifier and software than ASD measurement (same conditions except for zoom*0, 65). The cutting was placed under the magnifier and moved sideways to take pictures of the entire cutting. About twelve to sixteen photos were usually needed to cover the entire cutting. Two and a half months after the infestation, reproduction of the first generation and fixation of the second generation had occurred. It was therefore possible to repeat the counting of female fixed on the same cuttings, to have more information regarding the WPS population dynamics.

2.5 Pollination experiment

2.5.1 Experimental sites and installation of the hives

The test consisted of adding bumblebee hives (*Bombus terrestris)* or red mason bee (*Osmia bicornis)* or horned osmias (*Osmia.cornuta)* shelters and measure the impact on fruit production. The experiment was set in in the fourteen farmer's orchards in Côte d'Or to take into account the diversity of local landscape contexts. A map showing the geographical location of each plot studied in the trial is available in the appendices.

Bumblebees (Bombus terrestris)

In six different NB orchards, a bumblebee hive (Natupol Booster, KOPPERT) was placed at 50m of the extremity of the orchard. A total of 17 branch ends were marked at different distances with fluorescent marking tape. The branches labelled were positioned at different locations compare to the hive: i) above the hive, ii) in the same row at three, nine, twelve and thirty meters, and iii) in the side rows compared to the hive at three, nine, twelve and thirty meters. (Figure 5). Ten additional branches as far as possible of the hive were marked as controls, at a minimum of 100m from the hive.

Osmias (Osmia cornuta, Osmia bicornis)

In eight other blackcurrant plots, an osmia shelter (Osmipro, POLLINATURE) of both species was installed, at least fifty meters from the "entrance" to avoid an edge effect. The two osmia shelters had to be spaced a hundred meters to avoid interference between species. Branch ends were marked at different locations compare to the osmia shelter: i) above the hive, ii) at three meters in the four directions. Finally, iii) a gradient was designed at six, nine, twelve and thirty meters from the hive in two directions (Figure 6). The control branches followed the same pattern. They have been installed as far away from the shelters as possible, at least a hundred meters away.

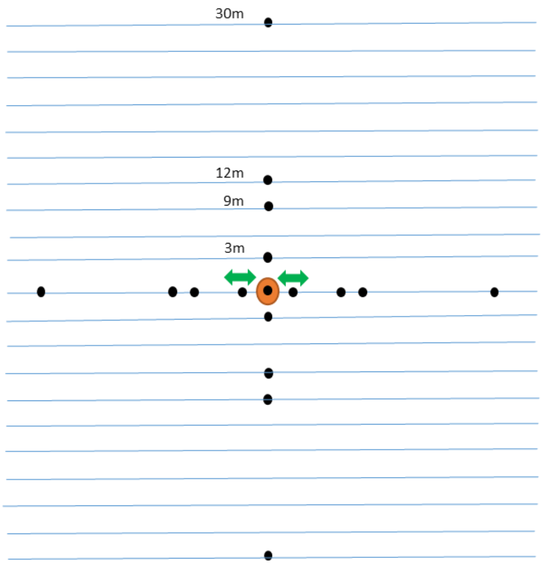

Figure 5 Scheme of the hive and gradient (marked branches) for the bumblebee experiment

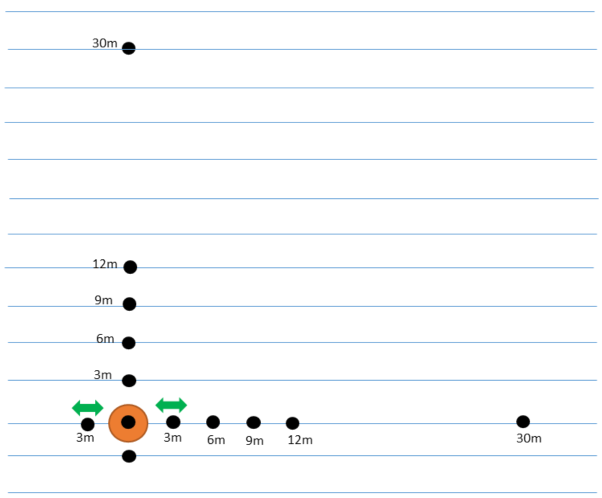

Figure 6 Scheme of the hive and gradient (marked branches) for the osmia experiment

2.5.2 Reproductive success with added pollinators.

Fructification rate, fruit initiation and fruit weight were measured on the extremity of the branches as in phenology measurement of the varietal collection (paragraph 2. 2. 1 Phenology) Since visual inspection of bushes suggested that the increase of pollination could be higher in the center of the bushes, we also counted, the number of inflorescences from the base to the label of the marked branches. (At that time fruit scars were no more easily detectable and we were unable to estimate the abortion rate). A few days before harvest we collected and weighted all the fruits produced on one meter of the foliar hedge, about 3m of the hives and on both sides of the hives. Same measurements were done in the control part of the plot with a total of 4 counts for bumblebee plot and 6 meters for *Osmia*.

2.5.3 Statistical analysis

Two Generalized Linear Mixed Models (GLMM, package lmer on Rstudio) were tested on the osmia and bumblebee dataset for the harvest results. The fixed effect was the treatment and the plot the random effect. The first GLMM was used to check whether there were significant differences between the volumes of bushes on different treatments and controls. If bushes were similar, the harvest per square meter could be expressed. This factor was used in a second GLMM to test the effect of the treatment on the harvested weight per cubic meter
For the marked branches, a GLMM was also used, following the binomial law as a flower can either have aborted or given a fruit. The results of the models provided an estimate of fruiting rate of the control and treatments, the percentage of flowers that will give a harvestable fruit. Finally, these rates allowed estimating the increase of the yield with the treatment. M. Duchet and MC. Anstett, CNRS performed this statistical analysis.

3) Results and discussion

3.1 Phenology

Classification according the early and late varieties

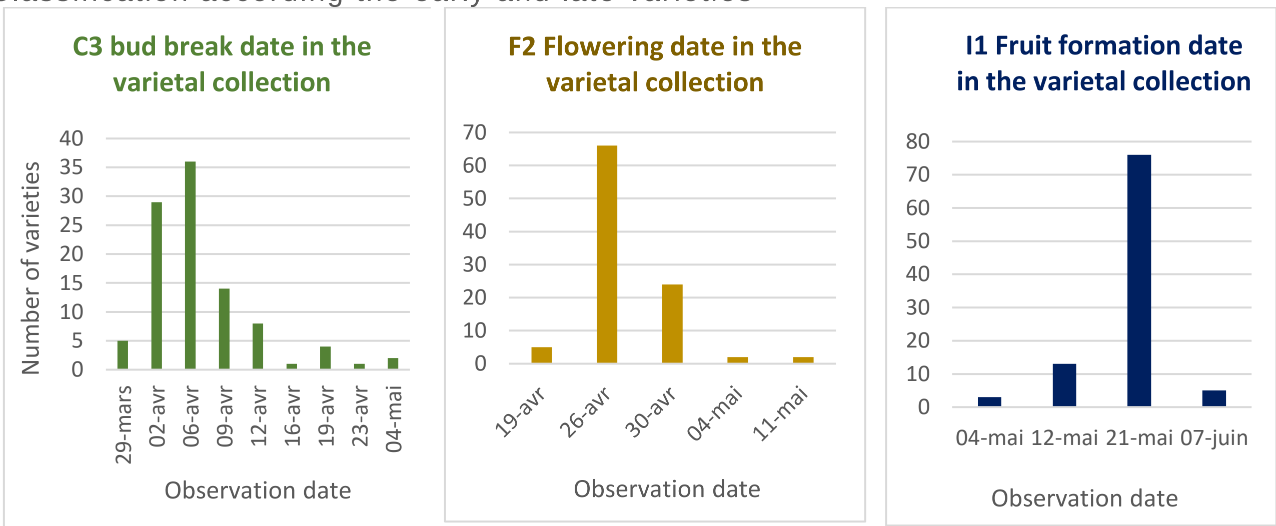

Figure 7 Chronology of the key phenological stages by varieties of the collection

Most varieties in the collection reached key phenological stages at similar dates: bud break (C3 stage) between April 2 and 9, flowering stage (F2 stage) between April 26 and 30, fruit formation (I1 stage) on May 21 (Figure 7). About ten varieties had followed a tendency towards precocity or lateness with one or two key phenological stages advanced or delayed compared to the rest of the collection. Some varieties can be identified as a very early such as Coronet or Tifon. On another hand, varieties like Ben Alder and Ben Tirran can be considered as the latest varieties in the varietal collection, with a bud break May 5th , a flowering mid-May and a fruit formation observed June 7th. (A detailed ranking of varieties according to their precocity is available in the appendices.)

Discussion

It would be useful to follow the flowering and fruiting of early varieties next year, to see if the abortion rate of flowers of these varieties is important as they may be subjected to late frost in April. In addition, the abortion rate will allowed checking if the formation of the fruit can be carried out before periods of drought. This is the main advantage in choosing an early variety and is particularly suitable in the case of plains where the heat has been important in previous years. The late varieties describe above came from Scotland and were developed by the James Hutton Institute. It had created a whole range of varieties of blackcurrants with a late varietal trait more or less important, because all varieties are result from crosses between Ben Tirran, Ben Alder, and other individuals close at the genetic level. This range of Scottish late blackcurrants can be interesting to avoid late frost problems during a key period: flowering in May often begins after the freezing period. It matches the climate observed in the “Hautes Côtes” of the Côte d’Or. A further step could be the evaluation of correlation between precocity and abortion rate

The meteorological station registered a record of cold for the month of April for 20 years (corresponding in blackcurrant early flowering period), a record of drought (water deficit of 60mm in full bloom), and a record of heat from June 1 to 15 (ripening period) (Denis 2021 ; Météo France). Blackcurrant must therefore adapt to large annual thermal amplitude in key periods. A plant adapted for continental climate, which present the largest annual thermal amplitude, could be interesting for blackcurrant in Burgundy to resist to frost and drought. Some varieties presented in the varietal collection are coming from Russia, or Eastern European countries (Poland, Hungary). Information regarding home country of the parental varieties should be consider in the plant-breeding program.

With meteorological data available and phenological stages data over several years (from “Bulletin de Santé du Végétal” or the varietal collection), it is possible to start to build a predictive model based on accumulated degree-days on blackcurrant. This kind of model would allow creating a decision support tool to help producers to anticipate the annual planning. A preliminary test has been done during this internship (with Rstudio, package CARET, adapted for a machine learning method) but it gave a low prediction rate. To improve it, the first thing would be to increase the amount and quality of data available. More data a predictive model has, the more accurate it is.

3. 2 Correlation between fruit initiation and ASD measurement

Fruit initiation rate observed at different dates (T2: beginning of fruit development date, and T3: full fruit initiation date) on marked branches in 2021 were compared to ASD of the parental varieties in 2020. Not surprisingly, fruit initiation at T2 and T3 were correlated (Pearson r=0.65 p=0.03), but visual inspection of the plot showed one outlier (indicated by a blue arrow, Figure 8) that corresponds to the Tiben variety.

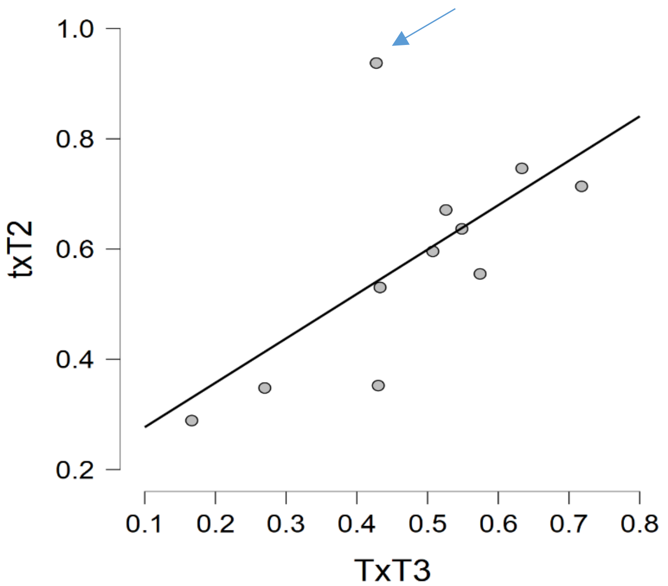

Figure 8 T2 and T3 observations of fruit initiation rate are correlated

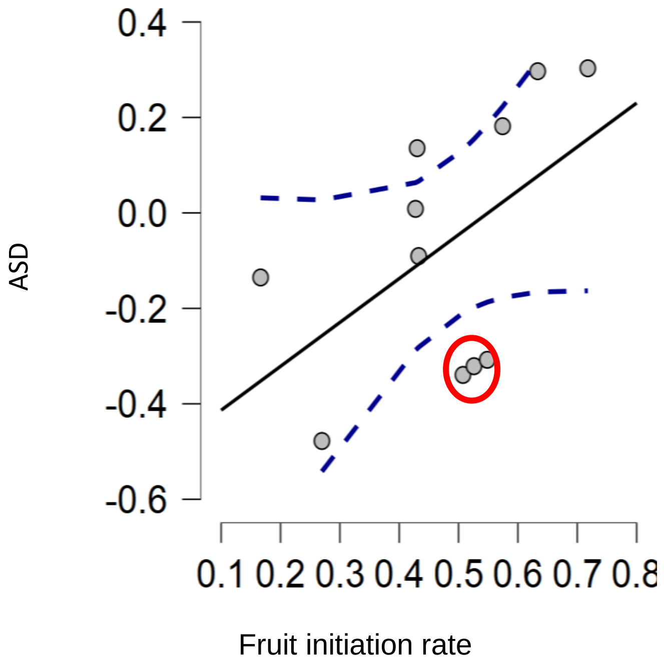

Figure 9 Fruit initiation rate at T3 and ASD confidence level on 11 varieties

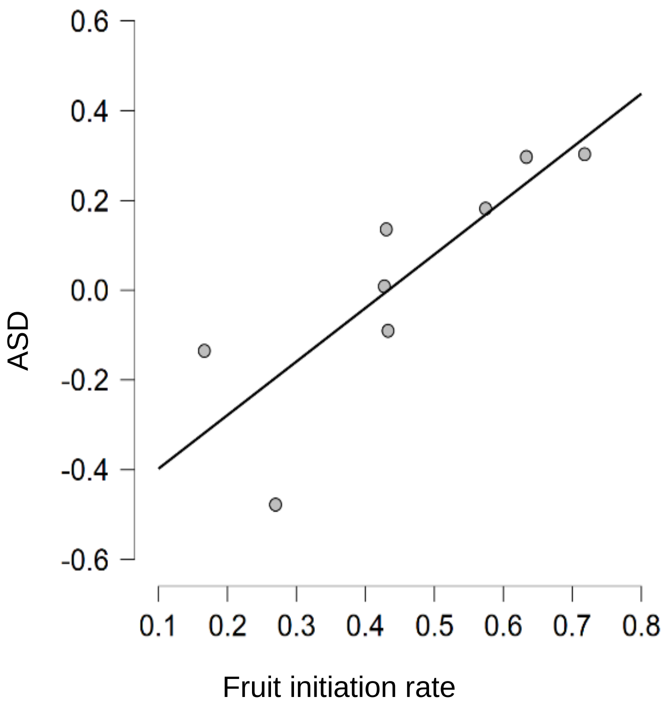

Figure 10 Fruit initiation rate at T3 and ASD on 8 varieties (without NB, RN, et Burga)

Overall, fruit initiation at T3 is correlated with ASD Pearson's r=0.52 but with a marginal significance p=0.09 (Figure 9). The result was the same on Spearman'rank correlation (Rho=0.55 p=0.082). Interestingly, the three varieties originating from Burgundy (Noir de Bourgogne, Royal de Naples and Burga) were clearly grouped (red circle, Figure 9). There had a low ASD (NB: -0,32+/-0,12mm, RN: -0,30)+/-0,18mm, Burga: -0,34+/-0,30mm) and a relatively high fruit initiation rate (NB : 52,6+/-14,2%, RN: 54,8+/-13,5%, Burga: 50,7+/-15,8%). There are out of the confidence interval. When removing these three varieties, the correlation turns highly significant. (Pearson's r=0.83, p=0.01). Correlation on ranks being even stronger (Spearman'n Rho=0.905 p=0.005) (Figure 10).

Burga is a mutant clone of NB, said to be more autofertile, and RN is known to be a self-fertile variety. However these two varieties have roughly the same ASD and the same style length as NB that would go against our hypothesis of a link between ASD and fruit initiation. Moreover these three varieties have a high fruit initiation in 2021. Generally, fruit initiation rate of NB variety is known to be lower than RN (Duchet, com. pers.) However on the other varieties, fruit initiation rate clearly increase with ASD. More work is needed to understand these results. A fruit initiation follow-up on hybrid F1 group next year could be interesting to continue the experiment and compare the correlation ASD and fruit initiation rate with the parental varieties.

3.3 ASD measurement on F1 hybrid

Results

Results of average ASD on each individuals of F1 hybrid from three crosses are presented below. On each figure, parents are represented in red, F1 hybrid in blue. The standard deviation is represented for each individuals.

NB * Ben Tirran F1 hybrid (short filament * long filament phenotype)

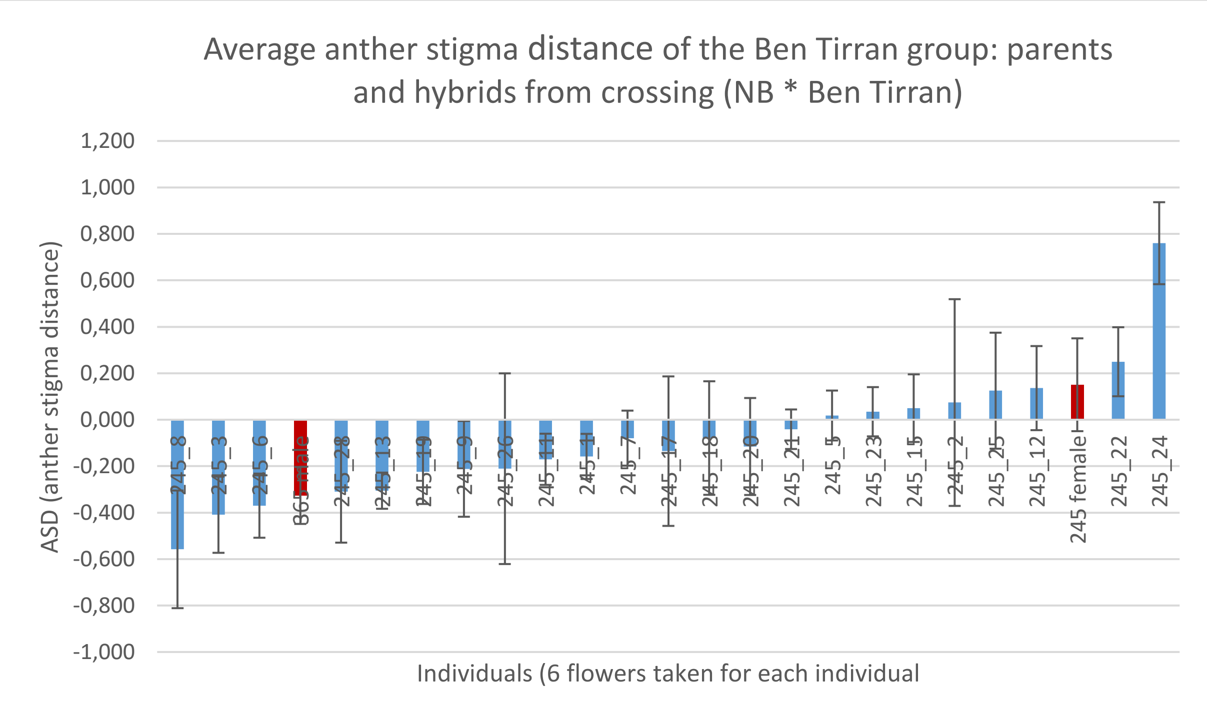

*Figure 11 Average anther stigma distance on NB*Ben Tirran F1 hybrids, parents NB (365) and Ben Tirran (245)*

There are 15 F1 individuals with a short filament trait (low ASD) and 8 individuals with long filament in the NB*Ben Tirran group. The ASD values differ from one individual to another, suggesting gene(s) controlling this trait are not homozygote. There are more individuals with a short filament phenotype in the group distribution. Hence, it is possible several genes are implied in the expression of this filament length trait.

NB * Lositkia F1 hybrids (short filament * short filament phenotype)

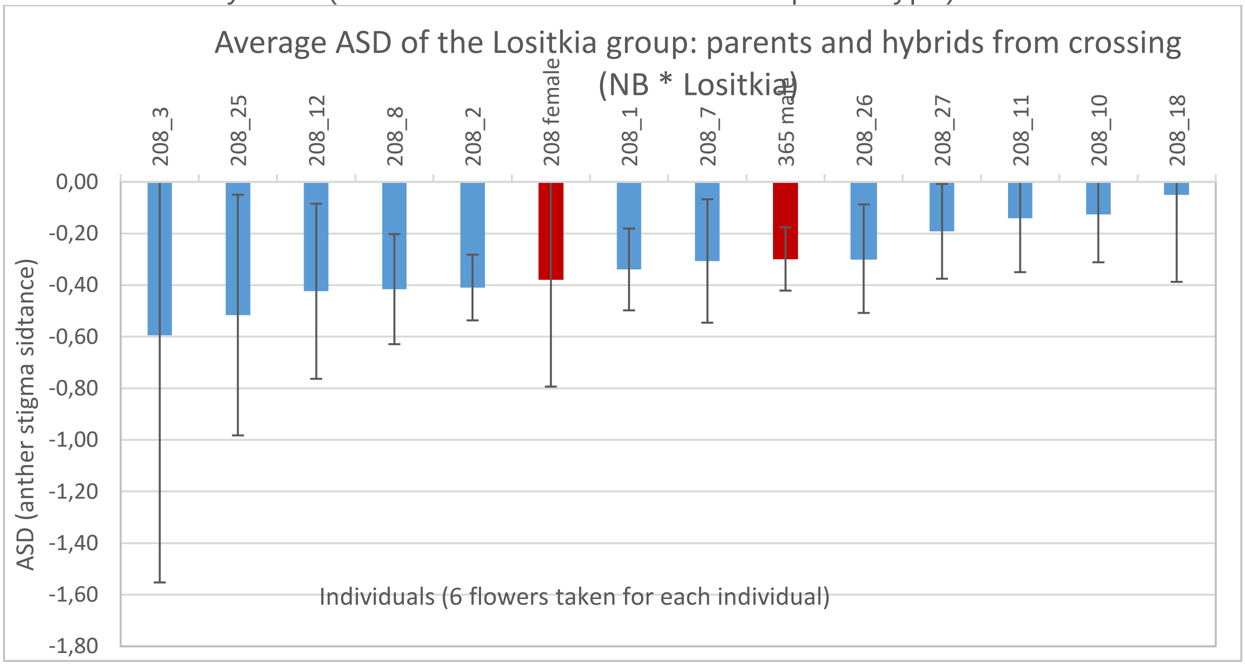

*Figure 12 Average anther stigma distance on NB*Lositkia F1 hybrids , parents NB (365) and Lositkia (208)*

In the case of NB* Lositkia F1hybrid, the two parental varieties have a short filament trait. The goal of studying this hybrid group was to see if there was a hybrid effect at the level of genetic determinism. In the present case, no phenotype of long filament emerges in the group. All hybrids share the same short filament trait with different ASD values.

NB * Andega F1 hybrids (short filament * long filament)

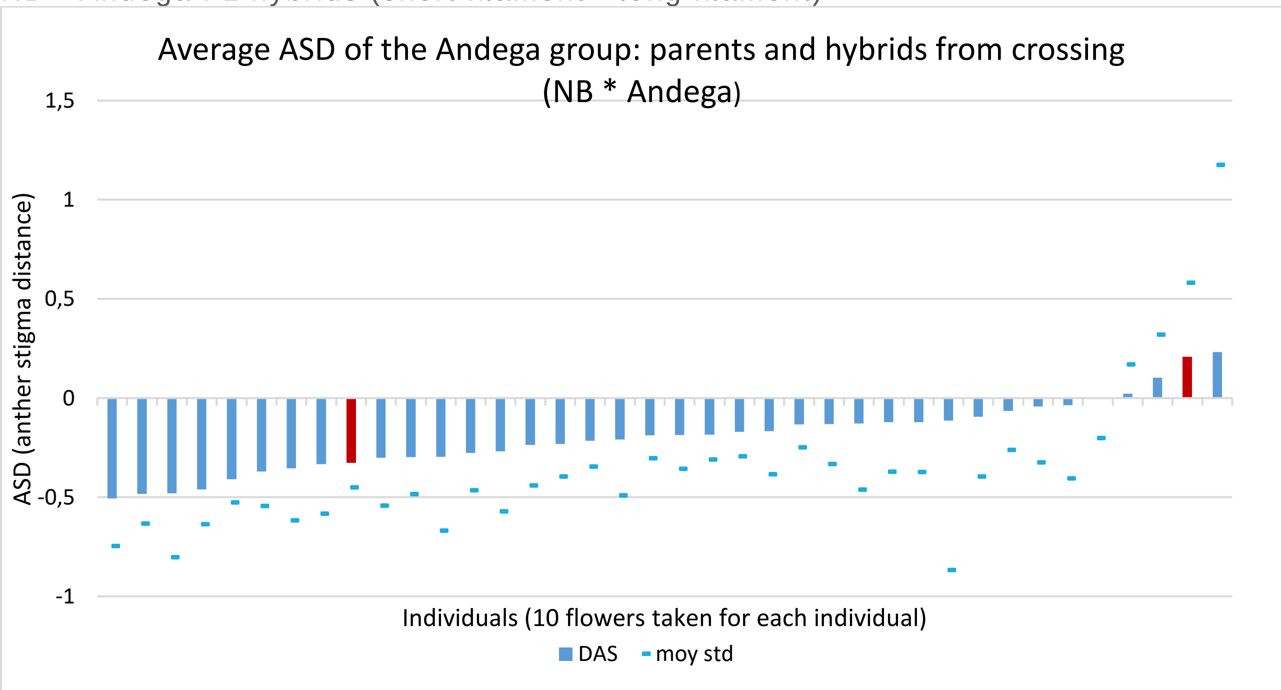

*Figure 13 Average anther stigma distance on NB*Andega F1 hybrids, parents NB (365) and Andega (242)*

A majority of NB* Andega individuals presented a low ASD, only three individuals have on average anthers above the stigma (Figure 13). This can be explained by a dominance of short filament in the NB*Andega.

Discussion: Comparison between hybrid F1 groups

Individuals of the F1 hybrid generation have an ASD value that differs from their parents. Within hybrid groups which have the short and long filament trait from their parents (NB*Andega, NB*Ben Tirran), ASD means between individuals are significantly different (nonparametric ANOVA, Kruskal Wallis test: NB*Andega χ^2= 117, df = 35, p-value = 9.099e-11, NB * Ben Tirran: χ^2= 79.493, df = 22, p-value = 1.963e-08). On the contrary, NB*Lositkia have a short filament trait from the two parents. This group showed ASD means between individuals significantly equal (Kruskal Wallis test χ^2= 19.556, df = 11, p-value = 0.05182). The filament length phenotype appears to be a continuous variable, without a threshold effect.

The dominance of the filament length phenotype seems dependent on crosses. Dominance of short filament phenotype seems more important in NB*Andega than NB*Ben Tirran group. There are 3 individuals with long filament phenotype in NB*Andega group (for a total of 37 individuals in the group) compared to 8 individuals of NB*Ben Tirran (for a total of 23 individuals in the group). This difference could be explained by the parental genotype between Andega and Ben Tirran varieties. Andega variety was obtained by crossing NB and Ojebyn (plant breeding INRA). Ben Tirran was obtained by crossing with Ben Lomond x Seabrook, Scottish and UK varieties genetically distant from NB. Andega's genetic background is therefore much closer to NB than Ben Tirran. NB having a short filament, it is coherent to see its strong influence in the NB*Andega group.

The standard deviation values are very large between flowers of the same individual; this does not allow us to conclude on the choice of F1 individuals to cross for the plant breeding program. However, the 8 individuals of NB*Ben Tirran with positive ASD should be considered for their long filament trait compared to other individuals of their group. They presented on average anthers above the stigma and does not have the supplementary NB background that can be presented in NB*Andega individuals.

This experiment will continue by the measurements on the others F1 hybrid crosses to confirm these first results. As the filament length phenotype seems to be controlled by several genes, it could be interesting to continue the experiment at the genetic level with a mapping QTL (Quantitative Trait Loci) method. This QTL method could allow looking for trait engaged in the filament length trait, and other characteristics of self-fertility trait.

3.4 Development of resistance test to WPS

Results

A problem with the cutting development in the climatic chamber had been observed in the development of infestation test. From 160 cuttings, only 55% have budded. Two months after repotting, only 45% of the cuttings showed healthy leaves. This poor development conditions affected all varieties. The main explanation put forward was the temperature: it was not possible to regulate the room temperature. Unfortunately, the temperature logger has malfunctioned during the test to confirm it. The number of healthy cuttings available for infestation were reduced. Some cuttings selected for infestation were not in good condition, especially the replicates of Troll and Royal de Naples cutting (Table 2).

Table 2 Condition of cuttings used for infestation tests

Variety	Conditions of the each replicate	
	Replicate 1 (R1)	Replicate 2 (R2)
Troll	Withered leaves ✗	Withered leaves ✗
Noir de Bourgogne	Good ✓	Good ✓
Royal de Naples	No bud break ✗	Withered leaves ✗
Leandra	Good ✓	Intermediary ≈
Ontario Climax	Good ✓	Withered leaves ✗

It was decided to count only adult females present on bark cutting. Males were present for a very short time period, and they were less representative of the infestation success. Moreover, it had been decided not to count the juvenile stages because they were hide under the bark, and it was sometimes difficult to differentiate a WPS juvenile stage from a drop of essential oil of the cutting.

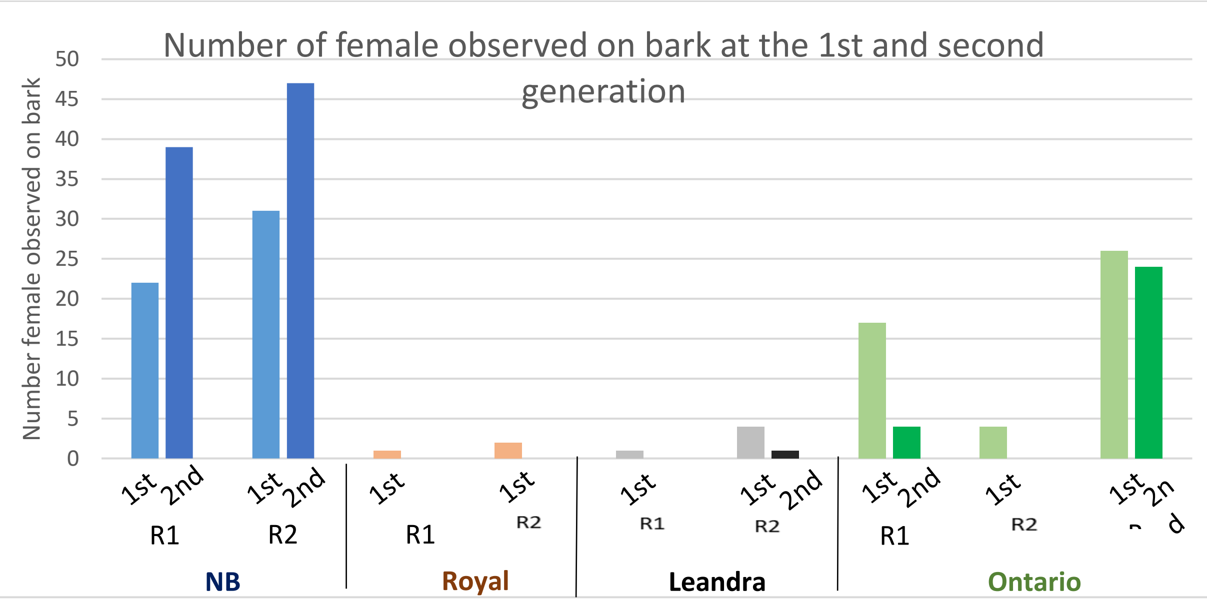

Figure 14 Number of WPS females observed on bark per plant, at the first (1st) and second (2nd) generation. R1, R2 : Repetition 1 and 2

At the first generation, there were five female shields (1 and 4 for replicates R1 and R2, Figure 14) set to Leandra bark, a variety known for its resistance to WPS, according to the observations of the varietal collection made in previous years. On the contrary, Ontario Climax, a variety known for its susceptibility had forty-three shields counted on the three replicates. Therefore, the trend of these controls seems correct. Fifty-three female shields were presented on the two replicates of the variety NB at the first generation (22 and 31 females on R1 and R2 cuttings, Figure 14). Only three females were observed on Royal de Naples (1 and 2 females for R1 and R2 cutting). This result can be explained by the poor condition of the cuttings. (Table 2). At the second generation, eighty-six females were fixed on NB cuttings (39 and 47 on R1 and R2). Thus, it is likely WPS female presented at the first generation are developed and laid eggs on cutting.

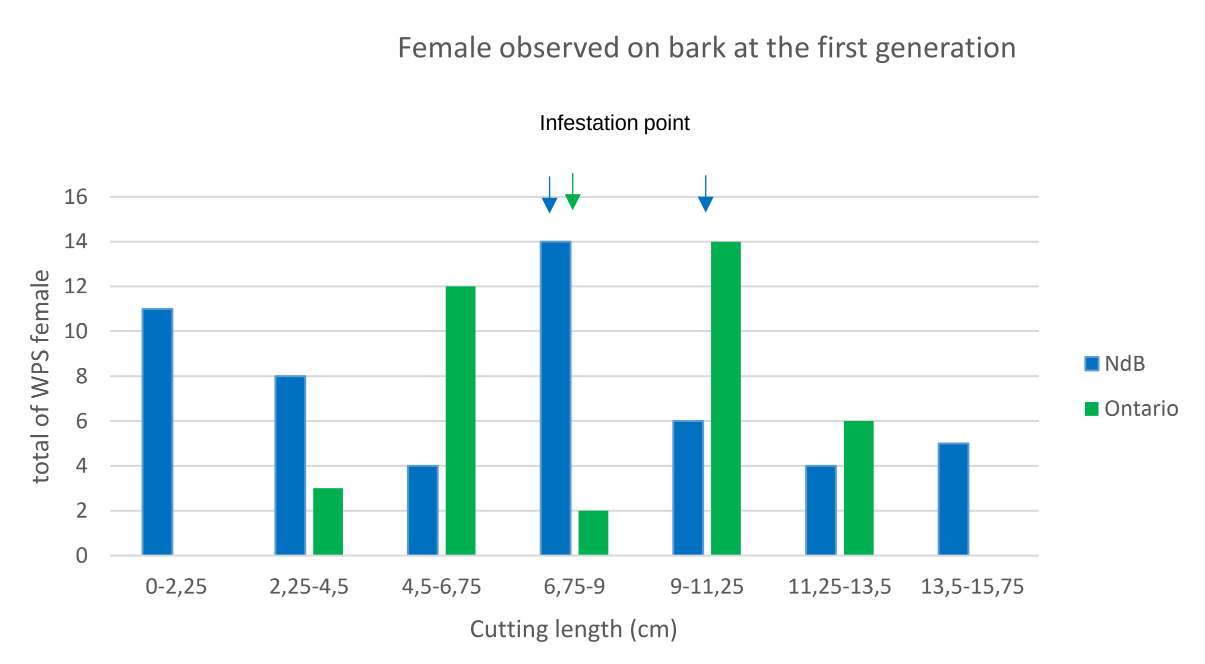

Figure 15 Location of WPS females on bark depending on cutting height

The figure 15 presented the total number of female observed on cuttings per variety, depending on WPS location on the cutting. The cutting length shown in the Figure 15 represents the length covered by a photograph under a microscope (zoom*0.65). The infestation points (where the egg deposit was made) were located between 6,75 and 9 cm for the cutting base for Ontario Climax, from 6,75 to 11 cm for the NB cuttings. Crawlers moved up and down on the cutting from the infestation point. On Ontario Climax cuttings, crawlers did not stay in the same area of infestation, but made short distances up and down the cutting (only 2 WPS females observed at the infestation point [6.75-9cm], while the 12 and 14 WPS females were located on [4,5-6.75] and [9-11.25]). The distribution of WPS females on NB cuttings was more spread over the entire length of the cutting, with a maximum 14 WPS shields were observed at the infestation area [6.75-9cm]. 11 WPS females were located at the bottom of the NB cutting ([0-2.25] section).

The development of this test had helped to highlight some surprising observations in particular concerning the WPS "behavior " which did not always match with literature or field observations. For example, some females were fixed to leaves (instead of fixed on wood for survival of the population year-to-year). Some females fixed to the bark came out of their shield. They were still alive, because their stylet still was planted in the wood, allowing them to feed.

Discussion

Due to the time required for each stage of the infestation (at least two months to have the first answers), linked to the low number of healthy cuttings available, the infestation tests could be done on a limited number of replicates. This lack of repetitions makes it impossible to draw clear conclusions, but allows to note trends and to give instructions to improve the infestation technique.

The tendency of the susceptibility test seemed correct as the most susceptible varieties (NB and Ontario Climax) had been severely attacked while the more resistant variety (Leandra) was less infested by female WPS. To confirm this trend, it is necessary to continue the test with a greater number of repetitions. This would make it possible to test statistically intra-variety repeatability and differences between varieties. The crawlers movement according to the variety must also be repeated. NB seemed to either stay on infestation point or go down to the base. It is surprising considering WPS nutrition. A study show WPS feed probably on photo-assimilates of phloem sap (Mizuta et al. 2004). Therefore, the food source is located in leaves, at the top of the cutting, and goes down to roots through phloem. The main proposal put forward to explain the choice of WPS to go to the base of NB cutting was the presence of a label at the base of the cutting. This label could created a microenvironment with more humidity and darkness, two favorable conditions for WPS development. It would be useful to remove it for the next infestations to verify this hypothesis. Moreover, leaves quality should be take into account to select good replicates.

Another possible improvement could be the diversification of laboratory's WPS rearing. The current WPS population used for the test comes from a single sample taken in the field in 2020 and developed at each generation. It would be interesting to renew the rearing in order to bring more genetic diversity.

These infestation tests are very experimental and poorly documented in the literature. A study examining the resistance of tea cultivars to WPS infestations showed that the duration of the developmental stages varied between resistant and susceptible cultivars: some stages were significantly retarded when reared on resistant cultivars (Mizuta 2005). It was not possible to verify the duration of each stage for this test, especially during the development of the first generation. However, it could be an interesting direction to continue the experiment. Moreover, a diversity of shield sizes were observed in this test, it would be a good idea to measure and compare shield sizes to check if there is a significant difference between varieties

3. 5 Pollination

Results

Volume of bushes harvested did not differ between control and added hives or shelters (*Osmia bicornis*: t=-0.623, df=38, p>0.5; *Osmia cornuta:* t=-0.524, df=38, p>0.5 *Bombus*: t=1.555, df=17, p <0.14).

The weight of blackcurrant harvested per cubic meter was different for the addition of *Osmia bicornis* 0.8798 ± 0.1384kg (t = 4.40, df = 38, p <10-45), and for the addition *Osmia cornuta* shelter 0.7421 ± 0.1384kg (t=1.98, df=38, p=0.05411) compared to the control 0.6286 ± 0.1384kg. The weight of blackcurrant harvested per cubic meter did not differ with the installation of a *Bombus* hive 0.5476 ± 0.1162kg (t=0.779, df=17, p=0.44651) compared to the control 0.5205 ± 0.1162.

The fructification rate is significantly higher with *Osmia bicornis* (37.5% ; z = 2.096, df = 281, p = 0.03) than the control (35.6%) whereas there is no difference between the control and *Osmia cornuta* (35.1% ; z = -0.604, df = 281, p > 0.5). In the same way, the fructification rate does not differ between *Bombus* hive (33.7% ; z = 0.866, df = 149, p > 0.3) and control (32.8%).

Discussion

This difference observed between osmias species and bumblebee can be explained by the flight distance of the bumblebee. It can fly on 2-3 km (Petermann 2020). Hence, on blackcurrant orchards, it is possible *Bombus* have visited flowers close to their hive but also controls at hundred meters away in the same field. This problem was known at the start of the project, that why large orchards have been chosen for this experiment. However, variations on pollination due to this bumblebee dispersion was unknown.

Adding pollinators in the blackcurrant fields, especially *Osmia bicornis* or *Osmia cornuta* has a positive effect on fructification rate and final yield. It would be interesting to look at the combined effect of these two insects. During field observations, *Bombus* and *Osmia* seem to have different behaviors in the choice of flowers visited. It seems that *Bombus*, a large insect, tended to stay more on the branch ends, while smaller osmias were likely more in the center of the bush. A test with the two insect’s species could confirm or deny this tendency. It would also check whether the fruiting rate is improved or not (if the two insects are competing for the same flowers).

The effect of the distance to the hive or shelter (according to the gradient at 3m, 6m, 9m, 12m, and 30m from the hive) compared to the fruit initiation rate is currently being studied. This information may provide information on the action distance of pollinators. A posteriori, it could estimate the number of hives needed per hectare to increase pollination and final yield. The following steps of this test will also focus on elements of landscape context. The surroundings of blackcurrant orchards, whether it is a field of rapeseed, wheat or hedges seem to have a strong impact on the flight of bumblebees and their choice to pollinate blackcurrant orchards. In some cases, bumblebees will be more attracted to these other elements than to the blackcurrant flower. It is therefore important to set up ecological structures allowing pollinators to stay in the fields. For example, both *Osmia* species can be breed directly by farmers, using suitable insect hotels. Other experiments have been set up in the PEI to answer these questions regarding flowers attractiveness for pollinators. A preliminary work has been done on the importance of flower strips in inter-row. The choice of floral species is yet to be determined, since this mix of flowers chosen must attract bumblebees at the start of the season but not compete with blackcurrant during the key flowering period.

4) General conclusion and perspectives

ASD and fruit initiation seems likely correlated. It could be a step forward to select traits for plant breeding program, because the fruit initiation counting on a new hybrid generation could be replace by ASD measures on the parental individuals. However, this correlation was based only on eight varieties, nine repetitions per variety. This probable correlation should be completed with a larger number of varieties to confirm or deny it. Moreover, it would be interesting to repeat the fruit initiation counting on the three varieties surprising data (NB, RN, and Burga) to determine the source of systematic error.

The *Osmia* test was conclusive, with a increase of final yield. The next step will be understanding the range of action of *Osmia* pollination. Installation of Osmia shelters directly by producers could be a final purpose to this experiment. In a development perspective of an *Osmia* rearing, several points should be consider. For instance, two phenological events in *Osmia* mason bee lifecycle are described as critical: the timing of adult diapause in the autumn, and the timing of emergence in the spring (Bosch et al. 2008). These two points should be take into account with particular attention for rearing development.
Results of the *Bombus* test were not as conclusive compared to the *Osmia* one. The main explanation put forward was the important flight distance of *Bombus terrestris.* Even if the *Bombus* results were ambiguous, some producers were convinced of the effectiveness of bumblebees on pollination in their orchards. They testified that they saw numerous *Bombus* flying over the entire field during flowering period. We cannot conclude whether these are pollinators from added hives or already present in the surroundings. Nethertheless, it may be valuable to reintroduce this pollinator into orchards for ecological reasons, to rehabilitate the pollination ecosystem service for the agro-system sustainability. This decision will be really depending on the economical context, based on the cost of *Bombus* hives for producers.

The continuation of the pollination research axis could be focus to establish a link between ASD measures and pollination system. Position of sex organs can have a great impact for the mating biology of populations (Barrett 2002). As results on hybrids groups suggest, ASD seems to be a continuous variable of herkogamy. In animal-pollinated plants like blackcurrant, approach herkogamy (anthers below the stigma) is the most common type of herkogamy and it is associated with a broad variety of pollinators (Lázaro et al. 2020). Pollinators visit stigma first, before removing pollen from anthers. Another hand, reverse herkogamy (anthers above the stigma) is less common and it is supposed to be related to long-tongued pollinators (the most common is *Lepidoptera*) (Webb, Lloyd 1986 ; Barrett 2003). The *Osmia* and *Bombus* test studied here were based on NB flowers that present a high degree of approach herkogamy. It could be judicious to look at pollinators of blackcurrant flowers with a degree of reverse herkogamy (anthers above the stigma) to observe if there are pollinator differences.

In literature, the study of variation in herkogamy was mostly focus on its effect on self-fertilization and the limitation of this process (Parra-Tabla, Bullock 2005 ; Fishman, Willis 2008 ; Vos et al. 2012). However, the reduction of self-fertlization cannot be cannot be the only explanation of this continuous variation. (Kulbaba, Worley 2008). In some case, this variation could reflect a selection of different pollinator assemblages (Lázaro et al. 2020). Even if the subject is poorly documented especially on variations in type and degree of herkogamy, we can assume the restoration of pollination ecosystem service should be based on a diversity of pollinators. This diversity guarantees a better stability and resilience of the system, necessary in any agroecological model (Calame 2016).

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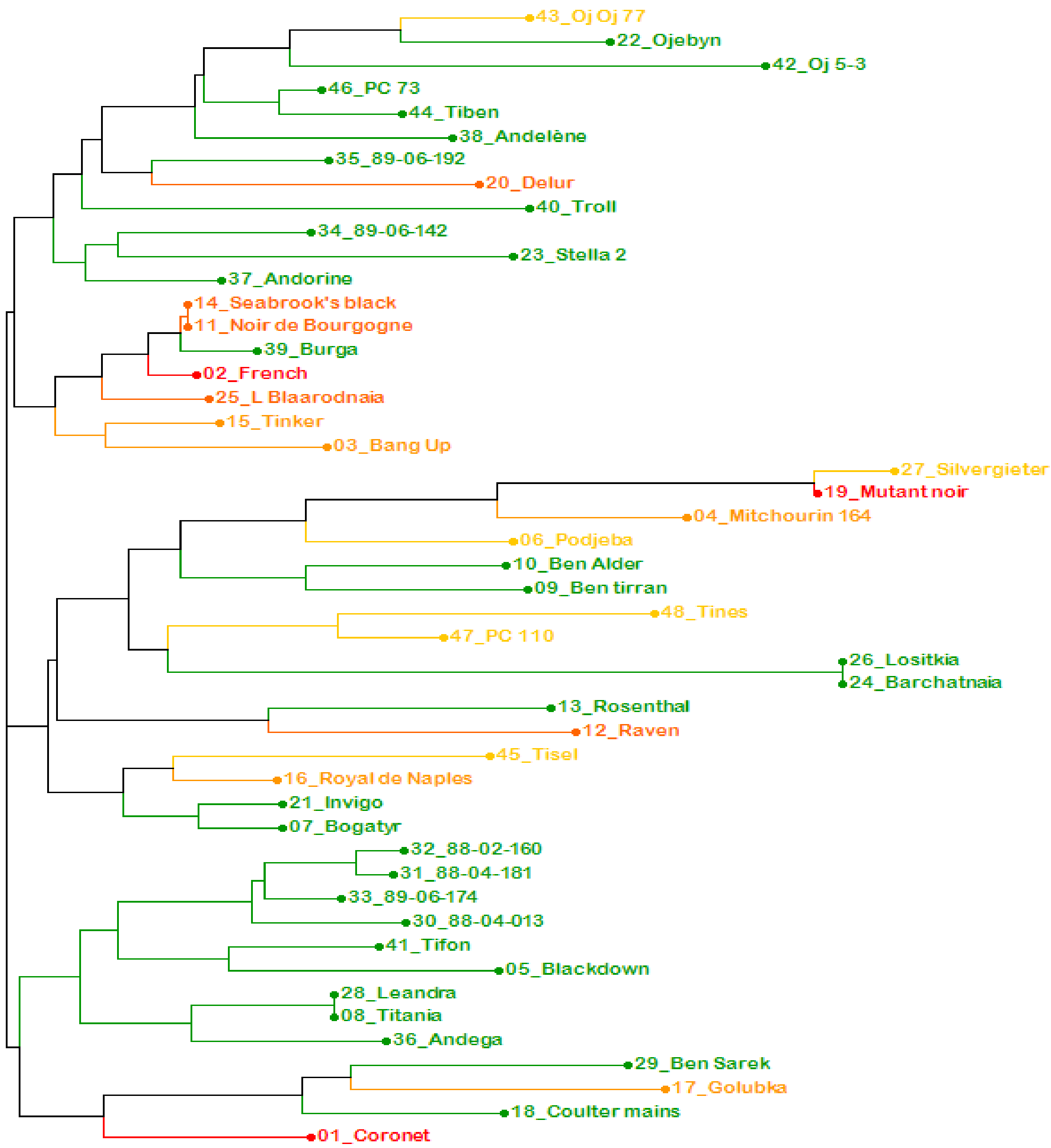
Appendices

Appendix I: Main phenological stages of blackcurrant development Source : Fabrice ECALLE, Chambre d'Agriculture Côte d'Or, 2018

Appendix II: Phenogram of 48 accessions from the blackcurrant collection obtained by the Neighbor-Joining method, based on the calculation of allelic dissimilarity distances for 8 SSR markers.



Appendix III: Classification of varietal collection 2021 according precocity ranking

Ranking	Description	C3 Bud break	F2 50 % Flowering	I1 Fruit formation
1	très précoce			04-mai
2	précoce-moyen	29-mars	19-avr	
3	moyen	06-avr	26-avr	
4	moyen-tardif		30/04 - 04/05/2021	
5	très tardif	04-mai		

➔ OLD VARITIES

Variety	Clone	Native country	Precocity 2021
Coronet	N 020-1	Canada	2
French	N 033-3 G	France	4
Merveille Gironde	N 051-3	France	4
Bang Up	N 081	UK	4
Mitchourin 164	N 128	Russia	3
Ontario Climax	N 129	Canada	3
Tenah	N 140	Netherlands	3
Ben Lomond	N 156	Scotland	3
Blackdown	N 158	England	2
Pobjeda	N 164	Russia	
Lissil	N 173	Allemagne	3
Bogatyr	N 197	Russia	3
Titania	N 227-1	Sweden	5
Ben Tirran	N 245	Scotland	5
Ben Alder	N 247	Scotland	5
Noir de Bourgogne	N 365	France	3
Raven	N 058-1	UK	3
Roodnop	N 059-1	Netherlands	4
Rosenthal	N061-1	Netherlands	3
Seabrook's Black	N 065-2D	UK	3
Tinker	N 069	UK	3

Variety	Clone	Native country	Precocity 2021
Champion	N 086	UK	4
Tor Cross	N 090-2	UK	3
Royal de Naples	N 094	France	3
Golubka	N 116	Russia	2
Pamjat Michurina	N 117	Russia	3
Coulter Mains	N 122	UK	3
Mitchourin 86	N 127	Russia	4
Mutant Noir	N 150	France	3
Delur	N 152		1
Record	N 153	Roumanie	2
Nachoka	N 167	Russia	3
Invigo	N 172	Allemagne	3
Meitgo	N 175	Allemagne	4
Ojebyn	N 181	Sweden	4
Stella 2	N 184	Sweden	2
Barchatnaia	N 194	Russia	3
L Blaarodnaia	N 207	Russia	4
Lositkia	N 208	Hugary	2
Silvergieter	N 212	Allemagne	3
Titania bis	N 227-1	Sweden	3
Leandra	N 238	Netherlands	4
Ben Sarek	N 239	Scotland	2

(Appendix III)

➔ VARIETIES COMING FROM INRA SELECTION

Variety	Clone	Native country	Precocity ranking 2021
88-10-075	N 585	France	3
88-07-042	N 588	France	3
88-04-013	N 589	France	3
88-04-181	N 595	France	3
88-02-171	N 597	France	3
88-02-185	N 601	France	3
88-02-160	N 602	France	4
89-06-174	N 613	France	4
89-06-142	N 616	France	4
89-06-092	N 617	France	4
Andega	N 242	France	4
Andorine	N 261	France	3
Andelène	N 380	France	2
Oj 10-5	N 306	France	4
Oj 3-3	N 312	France	3
82-24-085	N 378	France	3
88-07-087	N 517	France	3
82-16-079	N 523	France	3
Burga	N 185	France	3
Troll	N 240	France	3
Tifon	N 241	France	1
Oj 5-3	N 276	France	3
Oj Oj 77	N 322	France	4

➔ VARIETIES WITH EXPERIMENTATION LICENSE

Variety	Clone	Native country	Precocity ranking 2021
Tiben	N 644	Poland	3
Tisel	N 645	Poland	3
PC 73	N 646	Poland	3
PC 110	N 648	Poland	3
Tines	N 649	Poland	4
Viola	N 570	UK	3
Foxendown	N 637	England	2

➔ VARIETIES COMING FROM THE "CHAMBRE D'AGRICULTURE COTE D'OR"

Clone	Native country	Precocity ranking 2021
N 280	France	2
N 625	France	2
N 655	France	2
N 658	France	3
N 663	France	3
N 665	France	3
N 669	France	4
N 677	France	3
N 685	France	3

➔ POLISH VARIETIES

Clone	Native country	Precocity
PC 173	Poland	3
PC 425	Poland	3

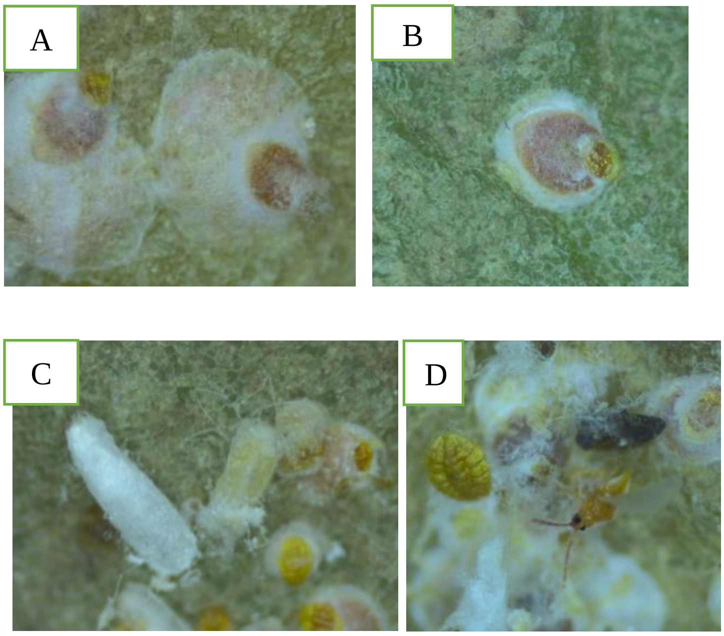
➔ PUBLIC VARIETIES

Variety	Precocity ranking 2021
Jet	3
Baldwin	3
Pobeda	3
Black Reward	
Tsema	3
Ben More	4
Daniel's September	3
23-08	3
21-64	3
365 (53 1G)	3
21-29	3

➔ SCOTTISH VARIETES (JAMES HUTTON INSTITUTE)

Variety	Native country	Precocity ranking 2021
Hedda	Scotland	(planted in 2020) 5
Ben Finlay	Scotland	(planted in 2020) 3
S36/1/101	Scotland	(planted in 2020) 3
S10/2/27/29	Scotland	(planted in 2020) 4
Ben Hope	Scotland	(planted in 2020) 3

Appendix IV: WPS Lifecycle stages observed in lab: A: adult female with her shield (the eggs are visible by transparency), B: Adult female, C: male and its cocoon, D: Male adult (M. Duchet, CNRS)



Appendix V: Map of the plot studied for pollination experiment, orange spots for osmia, and black spots for bumblebee hives

l'institut Agro agriculture • alimentation • environnement — AGRO CAMPUS OUEST	Diplôme : Ingénieur Spécialité : Horticulture Spécialisation / option : Agroecology Enseignant référent : Anne LE RALEC
Auteur(s) : Laura GREGOIRE Date de naissance* : 20/10/1996	Organisme d'accueil : SAYENS SATT Adresse : SAYENS SATT, Maison Régionale de l'Innovation, 64A Rue Sully, 21000 DIJON Maîtres de stage : Marine CHASSERAY-NARS et Marie-Charlotte ANSTETT
Nb pages : 35 Annexe(s) : 5	
Année de soutenance : 2021	

Titre français : Adaptation au changement climatique par l'amélioration génétique et l'optimisation de la pollinisation de la variété de cassis Noir de Bourgogne

Titre anglais : Adaptation of blackcurrant variety "Noir de Bourgogne" to climate change through plant breeding and pollination optimization

Résumé (1600 caractères maximum) :

La culture du cassis, notamment de la variété Noir de Bourgogne, est emblématique de la Bourgogne avec la fameuse liqueur de crème de cassis. Face aux difficultés de la filière pour maintenir le rendement, un projet d'innovation européen a été élaboré en association avec des partenaires scientifiques et techniques. Deux axes de ce projet sont développés ici. Le premier axe a consisté à rechercher des caractéristiques d'intérêt pour l'amélioration génétique du Noir de Bourgogne, afin de s'adapter au changement climatique et à une pression croissante de ravageurs. Il s'est décliné en plusieurs tests: observations phénologiques, suivi des floraisons et nouaisons, mesures de distance anthère stigmate et développement d'un test de résistance à la cochenille du mûrier *Pseudaulacaspis pentagona* sur des boutures. Le deuxième axe du projet est basé sur la restauration du service de pollinisation du cassis. Celui-ci a porté sur l'installation de ruches de bourdons et osmies dans les parcelles de cassis afin de mesurer l'efficacité de la pollinisation. A l'issu du stage, un lien entre distance anthère stigmate et taux de nouaison a été fait, des variétés ont été identifiées pour leur précocité ou tardiveté. Les principaux freins au développement du test de résistance ont été identifiés, et les premières infestations de cochenille en fonction de la sensibilité de la variété semblent avoir fonctionné. L'ajout de ruches de certains pollinisateurs est significatif par rapport à la pollinisation « naturelle ».

Abstract (1600 caractères maximum) :

Blackcurrant crop, especially Noir de Bourgogne variety, is emblematic of Burgundy, with the well-known liquor "Crème de cassis". Face to the sector's difficulties to maintain a crop yield, a European project was built in association with scientific and technical partners. Two research topics of this project were developed here. The first topic consisted in looking for traits for the plant breeding program of Noir de Bourgogne variety. The aim was to adapt the Noir de Bourgogne to climate change and increasing pest pressure. The first topic comprised several tests: phenological observations, flowering and fruit initiation follow-ups, measurements of anther stigma distance and the development of a resistance test to white peach scale (WPS) *Pseudaulacaspis pentagona* on cuttings. The second topic based on the restoration of the blackcurrant pollination service. It focused on the installation of bumblebee and osmia hives in blackcurrant plots in order to measure the effectiveness of pollination.

At the end of the internship, a link between anther stigma distance and fruit initiation rate was made, varieties were identified for their precocity or lateness. The main obstacles to the development of the resistance test have been identified, and the first infestations of WPS according to the susceptibility of the cutting variety seem to have worked. The addition of hives of certain pollinators was significant compared to "natural" pollination.

Mots clés : cassis, Noir de Bourgogne, distance anthère stigmate, pollinisation, cochenille du mûrier *Pseudaulacaspis pentagona,* amélioration génétique

Key words ; blackcurrant, Noir de Bourgogne, ASD: Anther Stigma Distance, pollination, white peach scale *Pseudaulacaspis pentagona,* plant breeding