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Artificial Pollination On Hazelnut In South Africa: Preliminary Data And Perspectives

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Abstract:

Hazelnut cultivation is rapidly expanding in regions outside its native range. New hazelnut plantations in South Africa are facing adverse environmental conditions which threaten the pollination process and hamper nut yield. Artificial pollination can increase fruit yield and fill pollination gaps in some fruit crops, but its application on hazelnut is still not well explored. This study investigated biological factors and technical procedures in the first artificial pollination experiment on hazelnut in South Africa. A suspension media for artificial application composed of 10% sucrose, 0,1% agar and 0,02% boric acid was used. In addition, alternative low-cost suspension media containing other forms of sugar were also evaluated. Moreover, a novel and practical approach to assess pollen conditions in liquid solutions was designed. Pollen viability was tested in the different suspension media. This varied greatly among the examined cultivars. Wild hazelnut produced the pollen with the highest viability, while Tonda Gentile delle Langhe (TGL) and Barcelona the lowest. Sterile grains were very abundant, especially in cultivated varieties (up to 65% in TGL). Preliminary data on nut yield were also collected. Altogether, this study indicated that artificial pollination is a promising approach for hazelnut cultivation in increasing the final yield. Further research is needed to develop integrated pollination strategies, especially in areas where the environmental conditions are adverse to the pollination process.

Keywords: *Corylus avellana* L., supplemental pollination, suspension media, pollen viability, field experiment

INTRODUCTION

Hazelnut (*Corylus avellana* L.) is a wind-pollinated and self-incompatible crop with winter flowering (Germain, 1994). Soil and atmospheric humidity are required to sustain pollen development and catkin elongation (Germain, 1994; Romisondo, 1977). Air humidity is also essential in maintaining stigmas hydrated as long as possible in order to maximize pollination success with compatible pollen. Recently, new hazelnut plantations were established in South Africa in a region with a temperate-warm climate (Conradie, 2012). Rain events are mainly concentrated during the long and warm summer, while extended dry periods are frequent during the short, cold winter. During winter, the daily average temperature and the solar radiation are still high compared to the usual climatic requirement of hazelnut. Furthermore, downslope winds often hit the area. The low atmospheric and soil moisture are detrimental for a proper development of both catkins and female flowers. Mimicking the natural process, artificial pollination proved to be a reliable procedure to increase fruit yield and to fill possible pollination shortages in adverse conditions (Pinillos

and Cuevas, 2008). In this study, a new procedure for pollen application on hazelnut in South Africa was developed and the associated biological factors were carefully controlled.

MATERIALS AND METHODS

Plant materials

Pollen was collected during winter 2016 in a hazelnut orchard located in Kwazulu-Natal region in South Africa, respectively from the cultivars Barcelona (S_1 S_2), Tonda di Giffoni (S_2 S_{23}), Tonda Gentile delle Langhe (TGL) (S_7 S_2) and from wild hazelnut plants commonly used as pollinators (*wild type*). Moreover, additional pollen was collected from wild hazelnut plants located in Hogsback (Eastern Cape, South Africa). The catkins collected from local plants were dried at room temperature overnight. The pollen was then aspired with the aid of a modified vacuum cleaner. This process was repeated at least twice. Finally, the pollen was stored at -20°C. In total, pollen coming from five different cultivars was analysed and two pollen donors (TGL and *wild type*) were selected for artificial pollination.

Pollen viability and germinability

Pre-hydrated and non-hydrated pollen was tested. The pre-hydration procedure was performed in a humid chamber at room temperature. Pollen was left in the humid chamber for at least one hour. To verify cell dehydrogenase activity, a small amount of pollen was slide-mounted with one drop of 0,1% 2,3,5-Triphenyltetrazolium chloride (TTC) and 60% sucrose solution (Volkmann and Rodriguez, 2006). In addition, to test cell membrane integrity a new approach was developed. PollenAid® (Kiwipollen, New Zealand, from here onwards PA), a buffer developed to maintain pollen viability of kiwifruit pollen in water-based suspensions, was diluted in deionized water. The PA solution was then mixed 1:1 with PollenAid Dye® (Kiwipollen, New Zealand), a non-toxic dye solution developed to mark pollinated plants in the field. It was noted that the dye does not stain viable pollen with an intact cell membrane, but stains those pollen grains with a disrupted cell membrane. To verify the reliability of this new method, *wild type* (*wt*) pollen was lab-treated in three different ways: (i) exposed to microwave for 30 seconds (i.e. killed), (ii) non-hydrated and (iii) hydrated. The membrane integrity was assessed in all the collected pollen samples. Finally, pollen germination of hydrated pollen was assessed on liquid medium (sucrose 0.2 M, PEG 0.04 M, H_3BO_3 1.6 mM, $CaCl_2$ 1.8 mM, $MgSO_4$ 1.7 mM, KCl 1 mM) utilizing the hanging-drop technique. Pollen tubes were then stained in 1% acetic carmine.

PollenAid® dosage and suspension media development

The novel method which assess the membrane integrity was also used to evaluate the correct amount of PollenAid® (PA) to mix in deionized water in order maintain pollen viability during the field applications. PA was mixed at different concentrations (3% as suggested in the PA label and 50%). These solutions, were utilized to slide-mount hydrated *wt* pollen, plus the dye (1:1 ratio). The same procedure was used to develop a pollen suspension media for artificial pollination composed of 10 % sucrose or sugar cane (as a cheaper alternative for field test in large scale and possible application at farm level), plus 0.1% agar and 0.02% boric acid. At the beginning, each medium was mixed 1:1 with the dye and a small amount of pollen was dispersed in a drop of the staining solution on a slide.

Pollen counting and viability scoring

The pictures of the slides containing the stained and the germinated pollen were taken combining a microscope Leica DM750 with an android phone (20.7 megapixel CMOS sensor) attached to the objective by a smartphone adapter. Images were then analysed with ImageJ software and the pollen was scored using the Cell Counter plug-in (Schneider et al., 2012). Two replicates of each sample were analysed and up to a thousand of pollen grains were counted for each sample.

Artificial pollination

Pollen from *wt* and TGL was applied on flowers of Barcelona cultivar. The experimental plot lays in an area without pollinators and far away from any source of fertile pollen. The pollen was either dispersed inside the pollen solution (PA or 10% sucrose, 1% agar and 0.02% boric acid) and sprayed on plants with common knapsack or applied on female flowers with brushes. Each treatment consisted of 30 g of pollen per hectare. Number of fruit set per each treatment was calculated.

RESULTS

All the tested cultivars showed strong male sterility with TGL having more than 50% sterile pollen grains (anomalous pollen without normal cell structures) (fig. 3). In Barcelona and Tonda di Giffoni male sterility was around 40% or above (fig. 1, 2, 3 and 4). Only in *wt* and in wild plants located in Hogsback, the sterility was reduced between 20% and 30%. Both dehydrogenase activity and membrane integrity benefit from the rehydration process. The TTC test showed a 5% increase in hydrated compared to non-hydrated pollen (from an average of 45.4% to 49.5 %) (Figure 1 and Figure 2), while the membrane integrity increased from the 2.1% in frozen pollen to the 35.6% in well hydrated pollen (Figure 5). On average, the pollen viability was higher with the TTC method (49.5%) compared to the dye-exclusion procedure (32,5%). The germinability showed the lowest values (23.1%). The results of the germination test weakly agreed with the results of the viability tests, especially in Barcelona and *wt* (Figure 4). Moreover, a strong variability in pollen viability and germinability between different cultivars and among different collection stages in the same cultivar was highlighted.

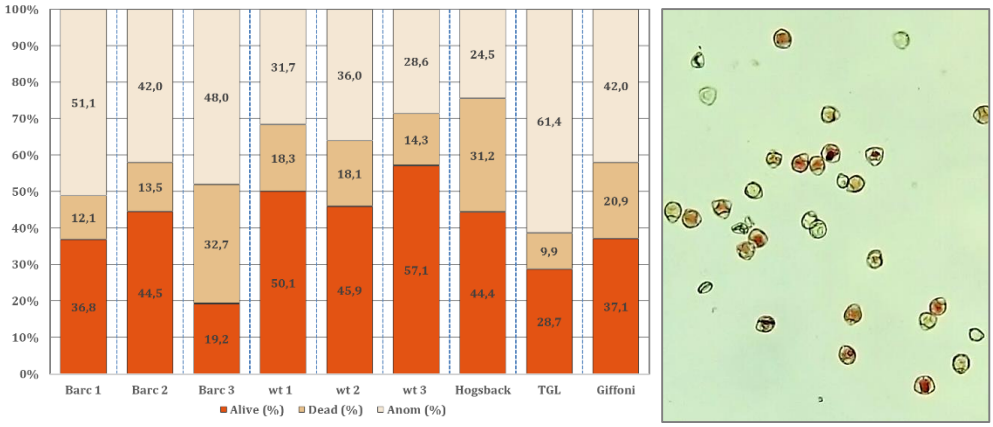

Figure 1. Viability as assessed with the TTC method and an example of the staining result. Pollen was incubated not hydrated. Barc = Barcelona, wt = *wild type*, TGL = Tonda Gentile delle Langhe. Numbers refer to different harvesting cycles.

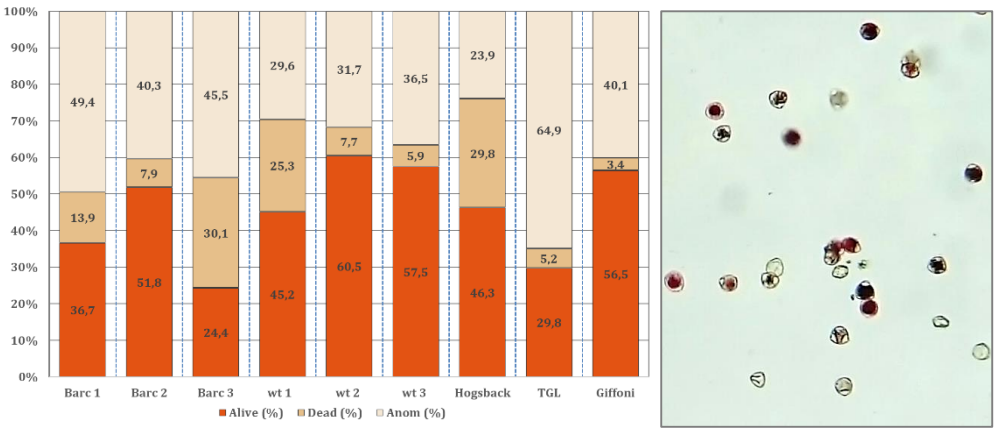

Figure 2. Viability as assessed with the TTC method and an example of the staining result. Pollen was incubated pre-hydrated. Barc = Barcelona, wt = *wild type*, TGL = Tonda Gentile delle Langhe. Numbers refer to different harvesting cycles.

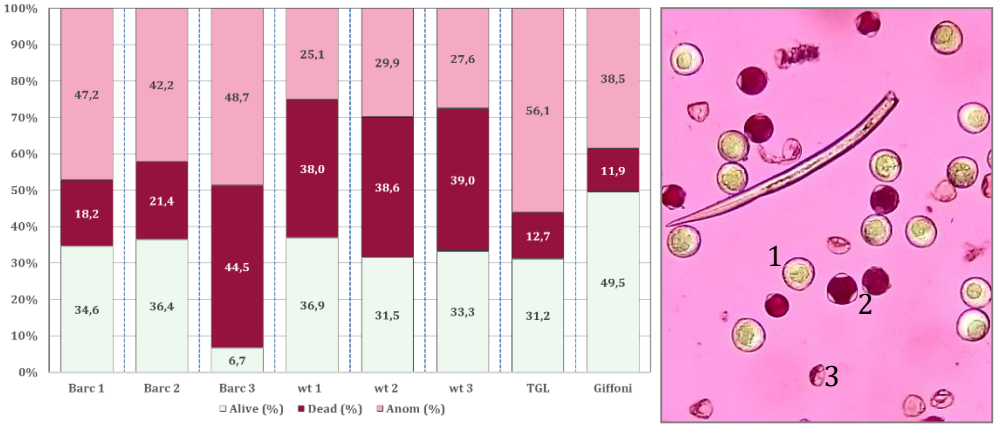

Figure 3. Viability as assessed with the dye exclusion method and an example of the staining result. Pollen was incubated pre-hydrated. Barc = Barcelona, wt = *wild type*, TGL = Tonda Gentile delle Langhe. Numbers refer to different harvesting stages. 1 = normal pollen grain alive, 2 = normal pollen grain dead, 3 = anomalous sterile pollen grain.

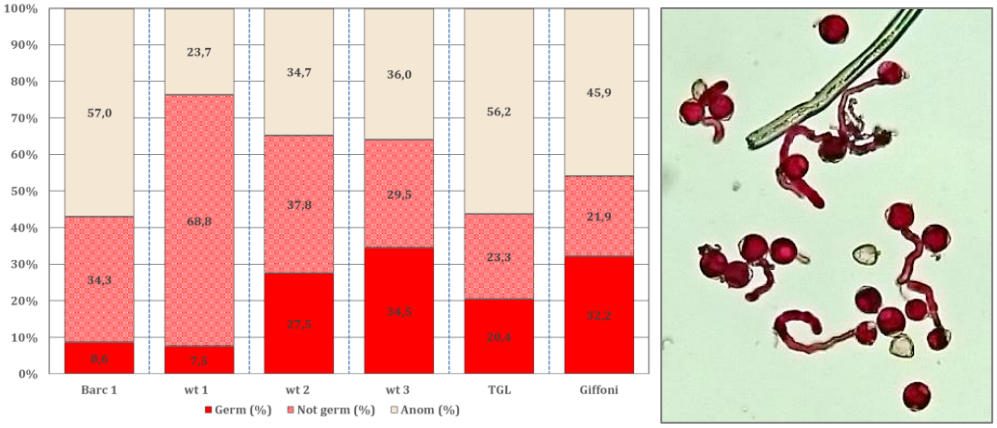

Figure 4. Pollen germination and an example of the staining result. Pollen was incubated pre-hydrated. Barc = Barcelona, wt = *wild type*, TGL = Tonda Gentile delle Langhe. Numbers refer to different harvesting cycles.

The reliability of the new dye-exclusion method gave good results as was able to differentiate between dead (microwave treated), non-hydrated and hydrated pollen (Figure 5). Furthermore, it was verified that the dosage of PA as advised for the kiwi-pollen was not enough to maintain the pollen viability in hazelnut. Dilution of 50% PA in deionised water gave the best results (Figure 5). The other tested media were able to maintain pollen viability as well as PA (Figure 5).

Figure 5. On the left: validation of the dye-exclusion method using *wild type* 1 pollen. On the right: effect of different media on pollen viability using Tonda di Giffoni pollen.

Finally, the pollen sprayed through a liquid solution (10% sucrose, 1% agar and 0.2% boric acid) gave better results than hand pollination alone (Figure 6). In fact, the pollen applied using a suspension media overcame the lack of moisture in the air. The hydrated pollen was sticking onto the stigmas of female flowers even if the stigmas appeared partially desiccated. In addition to the individual experiments of hand pollination and wet spray pollination, to investigate if there was a synergetic effect between these techniques, a separate experiment was conducted using hand pollination followed one week after by spray pollination. This last test gave the higher fruit set (weight of harvested nuts/average nut weight) compared to single applications, with +50% compared to the hand pollination and almost +20% compared to single application of pollen in a liquid solution.

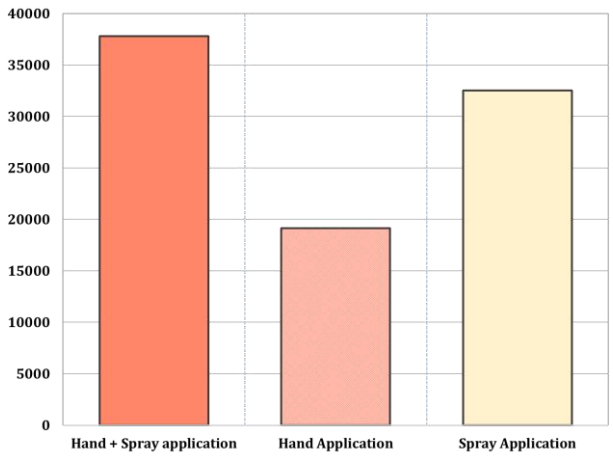

Figure 6. Number of fruit set (weight of harvested nuts/average nut-weight) per treatment.

DISCUSSION

Results indicate that artificial pollination is a promising approach in increasing the final hazelnut yield, when the species is cultivated outside its native range, and it has to face difficult environmental conditions, as low relative humidity, high solar radiation, high daily temperature and strong and dry winds during the flowering period. This result agrees with those found by Pinillos and Cuevas (2008) and support the fact that supplemental pollination techniques are useful when adverse environmental conditions affect pollen quantity and quality.

In artificial pollination experiments, both the control of different biological factors (i.e. pollen quality) and the application method are equally important (Pinillos and Cuevas, 2008). In this study, we could further ascertain that pollen of cultivated hazelnuts is strongly affected by male sterility, a phenomenon already highlighted by other authors (Novara et al., 2017; Potenza et al., 1994). Considering all the tests, the percentage of sterile pollen in Barcelona, TGL, and Giffoni is around 50% or more. This trait is usually diagnostic for translocation heterozygotes (Griffiths et al., 2015) and such chromosomic mutation has already been found in some important commercial cultivars (Salesses, 1973; Salesses and Bonnet, 1988). Research should thus focus on wild hazelnuts with low male sterility to increase pollination efficiency, while commercial cultivars are usually planted as pollinators. More efforts should be put into selecting hazelnut varieties able to produce good quantities of fertile pollen. Pollen viability can be affected by different factors, i.e. adverse abiotic agents, time and method of collection and storage procedures. In general, pollen viability increased after the hydration treatment. Pre-treatment of pollen in humid chambers is especially important to rehydrate cell membranes, as also emphasized by the new dye-exclusion method. Pollen germination tests highlighted lower percentage rates than viability rates assessed with TTC but similar to the results of the dye-exclusion test. The viability assessed with fluorescein diacetate (or FCR test) is also based on cell-membrane integrity and closely relates to pollen germinability (Hesse and Shivanna, 2003).

Considering the application method, in dry-winter regions, dry pollen supplements could be dispersed without reaching the target. The dry environmental conditions affected also the stigmatic surfaces of the female flowers and the fitness of the pollen granules. The pollen suspended in a liquid media, maintained the granules hydrated, gave important resources such as sugar and boron to boost the emission of the pollen tube, economize the pollen application rates and helped to moisturise the stigmas. The novel membrane integrity method proved to be essential in finalizing the medium. The solution should be composed of at least a sugar source and a stabilizer (Hopping and Jerram, 1980; Sakamoto et al., 2009). Two media were tested and both were able to maintain pollen viability.

Pollen applied through a suspension media showed to be much more effective than dry pollen applied by hand, showing the capacity of this technique to assist in harsh, variable and less conducive environment. Artificial pollination could be utilized in several cases on hazelnut, such as inadequate pollen source in the orchards, hot and dry weather threatening the pollen viability or desiccating parts of the flowers. Moreover, it could be one strategy to boost the natural pollination process, especially in years with adverse climatic conditions, helping to maintain an adequate production and productivity.

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