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HONEY BEE CONTRIBUTION TO 'BORDÔ' GRAPEVINE FRUIT PRODUCTION IN SOUTHERN BRAZIL[1]

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ABSTRACT- The production of fruits and seeds of many crops is increased when bees visit their flowers pollinating them. The aim of this study was to evaluate the effect of different pollination treatments on 'Bordô' grapevine (*Vitis labrusca* L.) fruit quantity and quality. Quantitative and qualitative fruit production parameters of plants visited by *Apis mellifera* L., manually self- and cross-pollinated plants and plants without pollination were analyzed and compared. Fruit production was high for all treatments and all fruits presented four seeds per fruit, on average, confirming that this grape cultivar is autogamous. However, fruit set after spontaneous self-pollination was statistically lower than that of all other treatments, and pollination by *A. mellifera* showed the highest fruit production. Furthermore, pollination by honey bees resulted in increased biomass, reflected on fruit weight, but the content of soluble solids remained unchanged. The results of this study showed that there is no need of pollinators for fruit production of 'Bordô' cv., but the presence of these agents, in particular *Apis mellifera*, influences commercially important quality parameters such as fruit yield and fresh weight. Therefore, the use of bee hives in areas with deficit of pollinating insects may promote an improvement in yield and quality of this cultivar.

Index terms: Self- and cross-pollination, *Apis mellifera*, *Vitis labrusca*.

CONTRIBUIÇÃO DA ABELHA MELÍFERA PARA A PRODUÇÃO DE FRUTOS DA VIDEIRA 'BORDÔ' NO SUL DO BRASIL

RESUMO- A produção de frutos e sementes de várias culturas é favorecida quando abelhas visitam suas flores, efetuando a polinização. O objetivo deste trabalho foi avaliar o efeito de diferentes métodos de polinização sobre a quantidade e qualidade de frutos da videira 'Bordô' (*Vitis labrusca* L.). Foram analisados e comparados parâmetros quantitativos e qualitativos da produção de frutos oriundos de visitas por *Apis mellifera*, de autopolinização manual, de polinização cruzada manual e sem indução de polinização. A produção de frutos foi elevada em todos os tratamentos, e foi observada a formação de quatro sementes, em média, por fruto, confirmando que a cultivar é autocompatível. Entretanto, a proporção de frutos formados por polinização espontânea foi significativamente menor em relação aos outros tratamentos e a polinização por *A. mellifera* apresentou a maior produção de frutos. Além disso, a polinização por abelhas resultou em aumento significativo da biomassa, refletido na massa dos frutos após a polinização, mas não na quantidade de sólidos solúveis nos frutos. Neste estudo, foi evidenciado que não há necessidade de utilização de agentes polinizadores para a produção de frutos da cv. 'Bordô'. Entretanto, a presença desses agentes, em especial de *Apis mellifera*, influencia parâmetros de qualidade comercialmente importantes, como a quantidade e a biomassa dos frutos. Assim sendo, o uso de colmeias de abelhas em regiões que apresentam déficit de insetos polinizadores pode promover melhoramento no rendimento e na qualidade desta cultivar.

Termos para indexação: autopolinização, polinização cruzada, *Apis mellifera, Vitis labrusca*.

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Wine production in Brazil has developed since the 19th century, when Italian immigrants started producing it mainly in the states of Rio Grande do Sul and Santa Catarina. Since then, most of grape varieties grown in Brazil were the American grapevine (*Vitis labrusca* L.), called as common (BRDE, 2005). Currently, the Brazilian wine-growing area is around 80,500 ha, with annual production between 1,400,000 and 1,500,000 tons and average yield from 18,000 to 19,000 kg/ha (IBGE, 2015). *Vitis labrusca* L. and its hybrids are the basis for the production of table wine and grape juice, representing over 85% the volume of processed grapes in the country (CAMARGO et al. 2010).

Ecosystems can provide several services when their full functionality is guaranteed, such as the regulation of natural processes, disease control and pollination, a service that has been intensely threatened (PALMER et al., 2004; KREMEN, 2005; KLEIN et al., 2007; WOOD et al., 2014; SANDHU et al., 2016). Currently, pollination is considered one of the key ecosystem services, given that humans depend on its association for food production, what makes it a process with relevant economic value (HANLEY et al., 2015). About 80% of flowering plant species depend on pollination by animals, such as bats, birds, butterflies, moths, wasps, beetles, flies, and especially bees (KEARNS et al., 1998; RICKETTS et al., 2008), and it is estimated that about 1,500 crops around the world require insects as pollinators (KLEIN et al., 2007; AIZEN 2009). Due to new market trends, winemakers are seeking to adapt new technologies in order to enhance and improve the quality of their products (PATERNIANI, 2001). Pollination has been characterized as an important process to improve the quality and quantity of fruits and seeds of about 70% of 1,330 tropical crops, increasing productivity and product quality, and combining agricultural production and environmental conservation (KLEIN et al., 2007). However, there are certain crops that produce fruits even without the participation of biotic pollinators, since their pollination is carried out by the wind (e.g. castor beans, coconut, canola, corn) or because they are autogamous, i.e. its flowers can be self-fertilized, such as beans and soybeans (FREITAS; NUNES-SILVA, 2012). Moreover, in most crops, productivity increases significantly in the presence of bees (ALVES; FREITAS, 2007; KLEIN et al., 2007). The reproductive strategies of *Vitis* species are highly variable, including dioecious, polygamous-dioic or hermaphrodite types. The flowers of some cultivars are fully self-compatible, some are self-incompatible and many are between these two extremes (FREE, 1993). Regarding grape pollination, different theories have been proposed, ranging from pollination by wind, insects or self-pollination, depending on the cultivar. Several studies have reported fruit production after spontaneous self-pollination, but there are controversies regarding the contribution of pollen vectors to the quantity and quality of these fruits (McGREGOR, 1976; FREE, 1993). For many species, there are few investigations regarding these aspects, despite the socio-economic importance of viticulture at local and regional level and the interest in improving production. The aim of our study was to evaluate the effect of different pollination systems on the yield and physical/chemical parameters of 'Bordô grapevine fruits (*V. labrusca* L.).

The study was conducted in two areas of a particular production of *Vitis labrusca* 'Bordo' cv. (L.) in the community of Palermo, Lauro Müller, southern state of Santa Catarina (28° 25'49.84''S and 49° 27'.06.68''W). The climate is Cfa, humid subtropical, with hot summers and no dry season (ALVARES et al., 2014). The soil of the region is clayey (CIRAM, 2002) and the vegetation has characteristics of a dense submontane rain forest (VIBRANS et al., 2013). In the first area, grapevines were grown from seedlings originated from the property by cutting propagation and in the second area they were grown from seedlings external to property. Grapevines in both areas were arranged in a canned system (horizontal canopy), spaced at 1.2 m x 3.0 m, with average age of five years. The experiments were performed between October 2013 and February 2014 and in the same period in the 2014/2015 season, corresponding to the flowering and fruiting time of grapevine. Anthesis was observed from 5 a.m. until the end of the anthesis with one-hour intervals in five flowers of ten inflorescences of five plants randomly selected. The average number of flowers per inflorescence and daily open flowers per inflorescence until senescence were estimated. In previously labeled and bagged flowers, the opening and longevity of flowers were determined and the period of stigmatic receptivity was verified using 3% hydrogen peroxide (KEARNS; INOUYE, 1993). In order to evaluate which pollination system is more effective on qualitative and quantitative parameters of 'Bordô' fruit production, different pollination treatments were performed in four newly opened flowers of five inflorescences of five grapevines randomly selected in the first area, totaling a sample of 100 flowers per treatment per year. Before each treatment, inflorescences were bagged in nylon bags in the pre-anthesis stage. The treatments performed were as follows: (1) spontaneous self-pollination, in which inflorescences remained protected with nylon bags without manipulation throughout flowering to prevent pollination by insects; (2) manual self-pollination, where flowers received pollen from the same plant; (3) manual cross-pollination, carried out by collecting pollen grains of flowers from the second area; (4) natural pollination, where flowers were not manipulated and left exposed to the action of all flower visitors and; (5) *Apis mellifera* treatment: monitoring was carried out through the removal of insects of other species so that only *A. mellifera* bees visited flowers. Fruits from different pollination treatments were harvested during the ripening period and, in the laboratory, they were individually weighed to obtain biomass. Subsequently, the content of soluble solids in fruits was measured, represented in °Brix and, the number of seeds per fruit was quantified. In order to evaluate the data set distribution, the Kolmogorov-Smirnov test was performed. As the normality assumption could not be met, the non-parametric equivalent of the analysis of variance, the multiple independent comparison and medians test of Kruskal-Wallis, was used (FIELD, 2009) to detect differences in fruit yield, biomass and soluble solids among fruits produced after pollination treatments. After observing the significance in the comparison between groups (pollination treatments), the non-parametric Mann-Whitney test for two independent groups was applied. All analyses were performed using the SPSS (Statistical Package for Social Sciences) software version 20.0 (IBM, 2011).

The flowering period took place in October lasting eight to nine days. On average, 22 ± 3 of the 71 ± 9 hermaphrodite flowers per inflorescence opened daily. The time of anthesis occurred at 9:30 am, extending to 12:00 p.m. During this period, flowers randomly opened in inflorescences. Androecium and gynoecium maturation occurred simultaneously since the flower opening and the longevity observed was one day. Over the day, anthers became dry with twisted filaments and the stigma remained receptive throughout the anthesis. Nunes et al. (2016) evaluated the floral biology of 'Isabel" cultivar and also observed the absence of dichogamy strategy in flowers. Regardless of pollination system applied, fruit yield was high and in all treatments, fruits presented an average of four seeds per fruit (Table 1). The high fruit production in treatments with spontaneous self-pollination and manual self-pollination confirms that 'Bordô' cv. is self-compatible. The fact that the stigmata were receptive and the opening of anthers occurred before the calyptra fall shows cleistogamy, a form of self-pollination reported in other species (MULLINS et al., 1992). Regarding the pollination of *Vitis* species, several theories have been proposed, varying from insect pollination to self-pollination, depending on the cultivar (PRATT, 1971). More recent studies have shown that most cultivars are self-compatible. Nunes et al. (2016) showed self-pollination for the *Vitis labrusca* 'Isabel' cv., corroborating results obtained for 'Bordô' cv. There is evidence of self-pollination in different *V. vinifera* (McGREGOR, 1976; HEAZLEWOOD; WILSON, 2004) and *V. rotundifolia* Michx. cultivars (SAMPSON et al., 2001).

After spontaneous self-pollination, fruit set was significantly lower than that of all other treatments ($p<0.05$), and pollination by *A. mellifera* showed the highest fruit production of well-developed fruits (97%), followed by manual cross-pollination (96%) and natural pollination (94.7%) (Table 1). Larger amount of fruits after pollination by insects compared to fruit production in bagged flowers was found in some cultivars with hermaphrodite *V. vinifera* and *V. rotundifolia* flowers by several authors (McGREGOR, 1976; SAMPSON et al., 2001; CHKHARTISHVILI et al., 2006), while other studies have not found increased fruit production (KELEN; DEMIRTAS, 2003; HEAZLEWOOD; WILSON, 2004). For *V. labrusca*, no research and/or confirmation regarding the influence of insect pollination on fruit yield was carried out to date. The manual cross-pollination treatment promoted fruit production similar to natural pollination treatment (Table 1), indicating that there was no shortage of pollinating insects in the study area during the study period. During flowering, *Apis mellifera* L. and occasionally some wasps were observed visiting flowers, which allow inferring that these bees are the main pollinators of 'Bordô' cv. Steshenko (1958) and Prior et al. (1985) reported the species as the main pollinator of *V. vinifera*, while Sampson et al. (2001) found species of Halictid bees as the most abundant flower visitors of this species. The results also confirm the existence of self-pollination and the qualitative and quantitative production gain appears to be dependent on biotic pollination, in particular on the service provided by bees (*Apis mellifera* in this study), one of the most important pollinating species associated with many species of wild bees (KLEIN et al. 2007, AIZEN et al. 2009).

Regarding the influence of different pollination treatments performed in our study on the biomass and °Brix of fruits, significant biomass differences between treatment with *Apis* and all other treatments ($p<0.001$) were revealed, and in °Brix between manual cross-pollination and the other treatments ($p<0.001$) (Table 1). Thus, pollination by bees resulted in increased biomass, which was reflected on fruit weight. A similar result was found for *V. vinifera* 'Cardinal' cv., which presented higher weight of fruits derived from natural pollination compared to spontaneous self-pollination (PRIOR et al., 1985), while Sampson et al. (2001) did not confirm differences in fruit quality after natural pollination and spontaneous self-pollination in *V. rotundiflora* Michx. This study showed that there is no need of pollinators for fruit production of Bordô cv., since its flowers are hermaphrodite and self-compatible. However, the presence of these agents, in particular *Apis mellifera*, influences commercially important quality parameters such as fruits yield and fresh weight. Therefore, the use of bee hives in areas with deficit of pollinating insects may promote an improvement in the yield and quality of this cultivar.

TABLE 1- Results of different pollination treatments performed in the *Vitis labrusca* cv. Bordô in the community of Palermo, Lauro Müller, SC.

Treatments	Number of flowers	Number of fruits (%)	Mean seed number	Mean fresh mass (mg)	°Brix
Spontaneous self-pollination	200	174* (87)	3.7	1.51	15.42
Manual self-pollination	200	180 (90)	4	1.51	15.00
Manual cross-pollination	200	192 (96)	4	1.50	14.99*
Pollination by *Apis mellifera*	200	194 (97)	4	1.53*	15.01
Natural pollination	200	189 (94,5)	4	1.51	14.99

* Significant difference from all other treatments ($p<0.05$).

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