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Insect controlled pollination in Japanese plum (*Prunus salicina* Lindl.)

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Abstract

Trials were performed in a commercial orchard of self-incompatible plum, whose pollination and fruit yield often are a real problem in this early blooming crop. For this reason we introduced in the orchard honey bees, mason bees, and bumble bees. The efficiency of pollinator insects was evaluated in the open field by counting—for 3 h a day in five sampling areas of the orchard—the number of visits to the flowers of the main cultivar. No bumble bees were detected visiting the flowers of the main cultivar. The other two pollinators showed a foraging activity which decreases by increasing the distance from their respective starting points. In each sampling area, the total number of visits was strongly related to fruit number and total yield. No significant differences were detected regarding fruit quality. Fruit yield of the most visited areas was very close to that obtained after mechanical pollination by pollen spray. Pollination was also performed on caged trees, using the different insects separately in order to evaluate their respective efficiency. © 1998 Elsevier Science B.V.

Keywords: *Apis mellifera* L.; *Bombus terrestris* L.; *Osmia cornuta* Latr.; Pollination; Fruit yield; *Prunus salicina* Lindl.

1. Introduction

The evolution of the angiosperms started and developed together with that of insects, and for this reason they are the most specialized pollen carriers from anther to stigma in a real insect–flower symbiosis (Tsyganov, 1953). As early as the end of the 18th century, the importance of insects as pollinators had already been acknowledged

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(Knight, 1799), and their role in plant pollination was pointed out by many researchers also in the last century (Darwin, 1889; Waite, 1895; Waugh, 1898). However, since the 1950s, crop protection from pests and weeds has proceeded by way of an indiscriminate application of potent chemicals, wherever agriculture is practiced (Plucknett and Winkelmann, 1995). This fact has unfortunately led to a widespread extermination of honey bees, wild bees and other useful insects.

Although many factors, such as climate, soil fertility, interplanting of proper selections, pruning, harmful insect control, adequate irrigation, etc. also affect crop yield, pollination is a very critical factor; no cultural practice will cause fruit to set if pollination is neglected (McGregor, 1976). The importance of an abundant pollination needs to be stressed, in fact, percent germination on the stigma and tube performance in the style strictly depends on a critical number of pollen grains 'delivered' to the stigma (Visser et al., 1988; Nepi and Lisci, 1996).

These general observations are valid for many fruit crops, but become dramatically important in relation to self-incompatible and very early flowering species, such as Japanese plum. In fact, although plum exhibits a wide range of incompatibility traits, from complete self-fruitfulness to complete self-sterility (Robinson et al., 1989), the most appreciated and highly demanded by consumers are the fruits from self-incompatible varieties.

Thus, in plum crops (whose yields vary considerably from year-to-year) two conflicting elements are simultaneously present: the absolute necessity for cross-pollination, and weather conditions during blooming time, which in early spring is often windy, cold, rainy and with low sunlight. In order to attempt to overcome these difficulties, we propose the use of insect pollination.

The present paper describes trials performed in an orchard of self-incompatible plum, where insects were introduced to test their pollination efficiency. Pollinator visits on the flowers were monitored, and the foraging activity observed in different areas was related to fruit yield. Our results seem to indicate that insects ensured adequate pollen delivery to the plum cultivar. Each of the species tested was able to perform cross-pollination contacts, as shown by experiments on caged trees.

Notwithstanding the possibility of improving colony performance and its foraging activity by using selected honey bee strains (Gordon et al., 1995), usually the weather conditions of early spring strongly limit the foraging activity of honey bees. Thus, the aim of this work was to study insect pollination on plum by means of other species also. Bumble bees, for example, exhibit a daily activity in the open field that practically complements that of honey bees and very rarely coincides with it (Benedek, 1996). On warm days, bumble bees fly mainly during the evening and resume their activity early in the morning (Prys-Jones and Corbet, 1991), while honey bees work mainly during the central part of the day.

A member of the Megachilidae of the genus *Osmia* could also play an important role in plum pollination. In fact, these insects like collecting nectar and pollen from flowers of the Rosaceae (Bohart, 1972; Asensio, 1983; Torchio et al., 1987; Pinzauti, 1992; Benedek, 1996); moreover, they seem to be quite well adapted to relatively low temperatures (Bosch, 1994a). For this reason, their flying period (February–April) overlaps with the blooming of plum.

2. Materials and methods

2.1. Plant material and orchard features

In 1996, trials were conducted in a 10-yr old commercial orchard of strictly self-incompatible Japanese plum (*Prunus salicina* Lindl.), cv. Black Diamond, located near Faenza (Ravenna, Italy); rows of cvs. Angeleno and Black Gold were also present (Fig. 1). Plants, grown as free palmette, were maintained under optimal conditions of pruning, fertilization, irrigation and protection by integrated pest management techniques. Rows were oriented in an E–W direction; tree density was 808 plants per hectare. The pollenizers were Sorriso di Primavera and the wild type Mirabolano, in a ratio of 1/8 with the main cultivar. During blossoming, the grass between and under the rows was cut to eliminate flowers of species other than plum.

2.2. Experimental plots

For open field pollination trials, five sampling areas, each consisting of five trees close to each other on the same row, were chosen to count insect visits and to evaluate fruit yield (Fig. 1).

In order to analyze separately the effects of different pollinating agents and of mechanical pollination, four other plots of three trees each were isolated by caging them under insect-proof plastic nets.

2.3. Pollinators

Insects were introduced at about 70% blossoming (April 5), both in the open field and under the nets.

2.3.1. Open field pollination

Pollinator colonies or nests were placed as shown in Fig. 1. The different insect charge per hectare, considering their different traits about pollination activity, was as after previous studies (Bosch, 1994b; Calzoni and Speranza, 1996; McGregor, 1976): eight colonies of honey bees (*Apis mellifera* L.), four colonies of bumble bees (*Bombus*

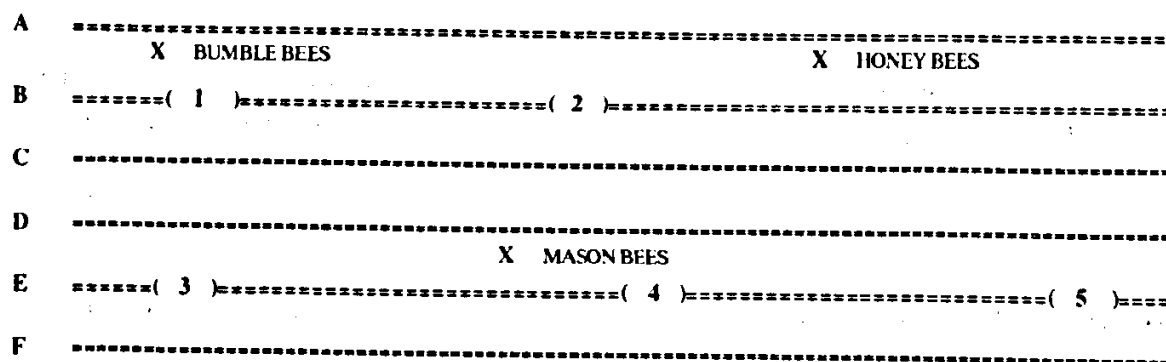


Fig. 1. Orchard map (not on scale) and sampling areas. A, B, E: Black Diamond; C, D: Angeleno; F: Black Gold. X: hive and domicile position; 1–5: sampling areas.

terrestris L.), and four artificial domiciles of mason bees (*Osmia cornuta* Latr.). The average number of insects was: 35 000 honey bees per colony; 200 bumble bees per colony, and 200 cocoons per domicile of mason bees ready to emerge.

2.3.2. Isolated tree pollination

Honey bees, bumble bees and mason bees were separately introduced under three of the caged plots. The insect charge per caged plot was as follows: about 8000 honey bees, 12 bumble bees, and 100 mason bee cocoons. It must be stressed that the dramatically higher number of honey bees was deemed necessary because of the well-known difficulties of this insect when it is not flying in the open air and in order to preserve the normal activity of the colony. 'Bouquets' of branches initially bearing unopened flower buds of the same pollenizers present in the open field were placed in water-filled drums under the nets of the isolated trees supplied with pollinators.

2.4. Pollen spray treatment

At about 100% blossoming, trees of the fourth caged plot were treated twice by spraying (using a 1.2 airbrush) with a freshly prepared 1:1 mixture of Sorriso di Primavera and Frontier pollen, collected in the same year and tested for high viability before suspending it in a liquid medium like in the work of Calzoni et al. (1979) (0.2 M sucrose, 3.2 mM H_3BO_3 , 1.3 mM $Ca(NO_3)_2$, pH 6.6) at a concentration of 300 mg/l.

2.5. Counting insect visits

Counting was performed at the same time in all the sampling areas for 3 h each day, i.e., 7 to 8 AM, 12 to 1 PM, and 4 to 5 PM. It lasted from the 4th day after insect introduction in the orchard, that is at about 90–100% blossoming (April 9), until almost all petals had fallen off (April 14). For each sampling hour and for each tree, insect visits on the flowers were counted visually (10 times of 3 min each per hour, at intervals of 3 min).

2.6. Recording of meteorological data

Climatic factors during the flowering period (temperature, relative humidity, wind speed, sunlight, rain) were recorded by a computerized multi-channel system (AD-1, Silimet, Modena, Italy), at a rate of three samplings every 2 s for each parameter.

2.7. Fruit evaluation

At harvest, total yield was evaluated in the five sampling areas and in the plots of caged trees. Moreover, a sample of 40 plums collected at random from each tree from each pollination treatment was used to determine fruit quality parameters such as average weight, size, and soluble solid content.

3. Results

Blossoming of plum in the year and in the area examined was about 3 weeks late compared with the average blossoming time of the last 10 years in the same orchard. Meteorological data, monitored throughout blooming, indicated that on the whole the weather had been always favourable to foraging activity of the insects. Table 1 shows temperature, relative humidity, sunlight and wind speed occurring when foraging activity of the insects introduced in the orchard was monitored. During blossoming, no bumble bees were detected in the open field on the flowers of the cv. examined. Thus, the results described henceforth pertain to honey bees and mason bees only (Fig. 2). Foraging activity from 7 to 8 AM was generally very rare. The visits were more numerous from 12 to 1 PM and from 4 to 5 PM. It is a well known fact that foraging activity increases with increasing temperature and sunlight, and with decreasing relative humidity. The foraging activity of honey bees was highest during the second sampling hour of the day. Mason bees, on the contrary, visited flowers with the same frequency during the second and third sampling hour. It rained only once during a sampling time, on April 11, 4–5 PM; during this period, foraging activity of both insect species became extremely rare or totally absent (Fig. 2). Moreover, on April 14, it drizzled during the whole day: the reduction in insect visits to the flowers is, however in this case, to be connected to the end of blooming.

In the open field, both mason and honey bees were seen while foraging pollenizers, simultaneous with their foraging activity on Black Diamond. Also on the pollenizers, visits were very rare or absent during the first sampling hour of the day, but frequent during the second and third.

The total number of visits on Black Diamond detected until complete petal fall and the relative length of insect flights are shown in Table 2. These data indicate that both pollinators visited flowers by flying across more than one row (Fig. 1). Within the same row (areas 3, 4, and 5), foraging activity of honey bees decreased at a linear rate with increasing distance from the starting points. Number of visits of mason bees (areas 1–5), by contrast, decreased with distance by a logarithmic trend. In both cases, a very strong correlation was observed (Table 2). Plotting the total number of visits of the two

Table 1

Average temperature, relative humidity, sunlight and wind during the count of insect visits

Day	Temperature (°C)			Relative humidity (%)			Light (μ E)			Wind (m/s)		
	a	b	c	a	b	c	a	b	c	a	b	c
April 9	12.5	20.5	20.5	79.0	48.5	53.5	364	1289	538	0.15	1.14	0.60
April 10	10.5	20.9	18.5	83.0	56.5	52.5	370	1280	554	0.37	0.70	1.98
April 11	10.2	20.0	15.2	82.5	40.5	72.0	427	1216	124	0.02	0.84	0.74
April 12	12.6	19.6	18.7	88.0	48.0	55.0	402	1509	528	0.15	0.83	1.49
April 13	13.2	21.2	21.2	68.5	53.5	40.5	400	1500	414	0.39	2.80	0.62
April 14	10.0	21.0	18.5	79.5	63.5	52.5	105	1526	668	0.76	2.41	1.06

a: 7 to 8 AM.

b: 12 to 1 PM.

c: 4 to 5 PM.

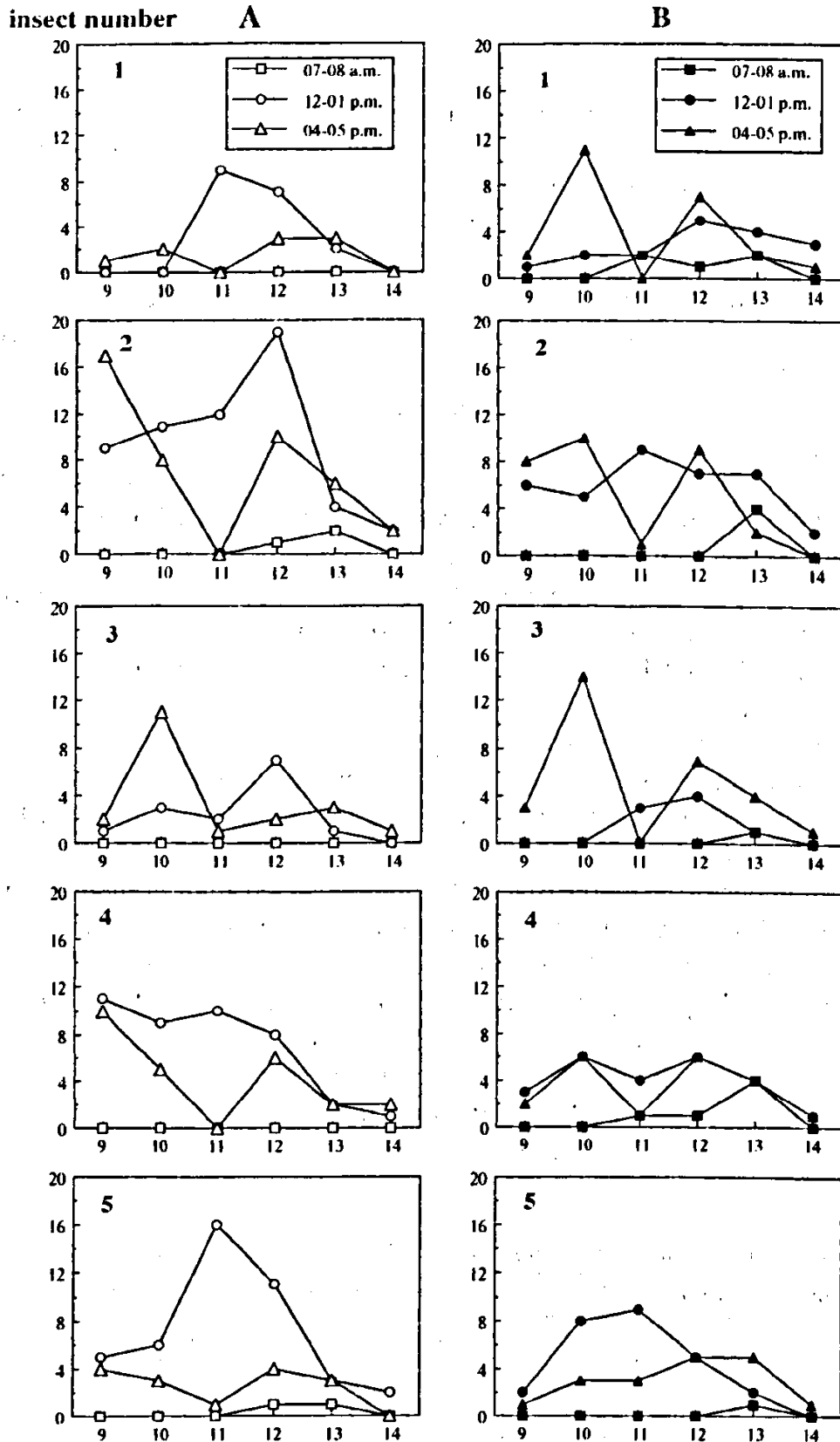


Fig. 2. Total daily insect number in each sampling area (1–5) counted in three different day hours in the open field. A: honey bees. B: mason bees.

pollinators vs. the number of fruits per tree in the different sampling areas reveals a strong correlation between these two parameters (Fig. 3). Moreover, a strong relationship between the total number of visits and total yield in the different sampling areas is

Table 2

Total insect visits (*a*) in the sampling areas and (*b*) mean distance of the area (m) from the hive or nest

		Sampling area				
		1	2	3	4	5
Honey bees	<i>a</i>	26	103	35	66	60
	<i>b</i>	120	48	121	29	51
Mason bees	<i>a</i>	42	72	35	50	38
	<i>b</i>	71	11	70	26	98

Regarding honey bees, visits in areas 3, 4, and 5 (on the same row) decreased in a linear trend ($y = 76.5 - 0.34x$; $R^2 = 0.99$) with distance from hives. Regarding mason bees, number of visits in the five sampling areas decreased with logarithmic trend ($y = 106.5 - 36.3 \log(x)$; $R^2 = 0.91$).

reported in Fig. 4; the trend clearly shows that above a certain number of visits, fruit production ceases to improve.

Data on the qualitative analysis of fruits at harvest are reported in Table 3. When comparing different sampling areas, no significant differences (Student's *t*-test, $P > 0.05$) were detected in sugar content. The fruit weight showed a high significant difference (Student's *t*-test, $P < 0.01$) when considering area 1 or 5 vs. areas 2, or 3, or 4. Fruit size was generally similar, ranging from 1st to 2nd class, without a close relationship with the number of visits.

Results on the yield from caged trees are also shown in Table 3. This trial was necessary in order to evaluate separately the respective efficiency of the pollinators, which otherwise is indistinguishable (or not easy to distinguish) in our experimental

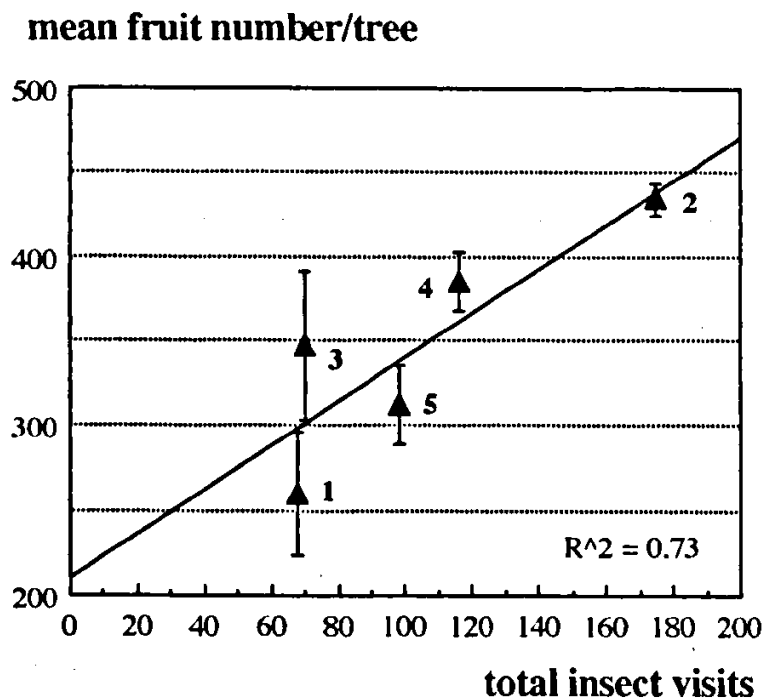


Fig. 3. Mean fruit number per tree after insect pollination in the open field; 1–5: sampling areas. Bars represent standard deviations. Highly significant difference (Student's *t*-test, $P < 0.01$) resulted when comparing area 1 vs. 3, or 5 vs. 4, or 4 vs. 2. Significant difference at $P = 0.05$ level resulted from area 1 vs. 5.

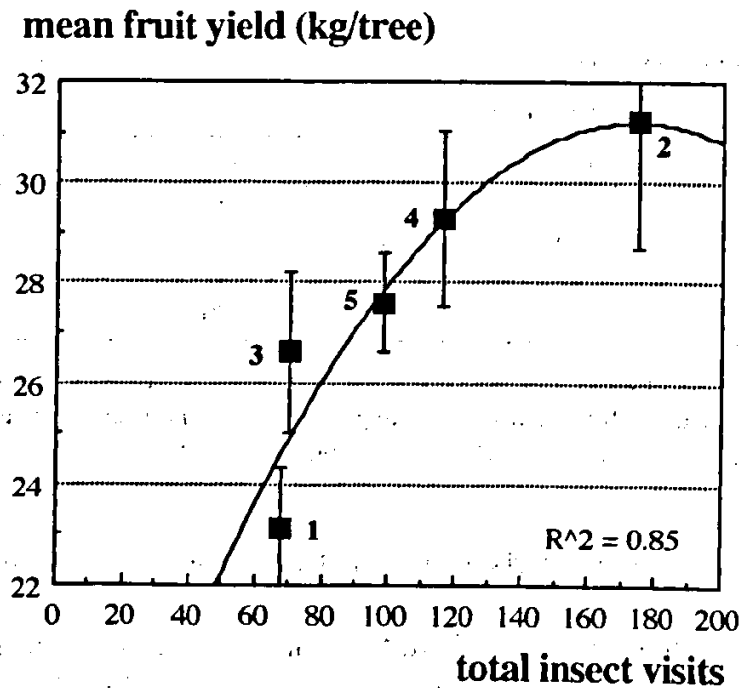


Fig. 4. Mean fruit yield after insect pollination in the open field; 1–5: sampling areas. Bars represent standard deviations. Highly significant difference (Student's *t*-test, $P < 0.01$) resulted when comparing area 1 vs. 3, 5, 4, or 2.

conditions in the open field. Therefore, although caging is an unnatural condition for both trees and insects, results from caged trees can reveal the individual efficiency of each pollinator. The total yield obtained as a result of honey bee pollination was unusually high, but it could be explained with the abnormal number of these insects introduced under the nets. On the other hand, it was very difficult to calibrate exactly the

Table 3

Fruit quality following insect controlled pollination in the open field and after different pollination trials on caged trees

	Fruit weight (g) (mean \pm sd)	Size (% per class)	Brix (%) (mean \pm sd)
<i>Sampling area</i>			
1	88.2 \pm 14.0	40/1	10.4 \pm 1.0
2	71.4 \pm 18.7	40/1	9.9 \pm 0.9
3	76.7 \pm 9.8	40/2	10.5 \pm 0.8
4	76.4 \pm 10.5	50/2	9.2 \pm 1.1
5	88.6 \pm 13.8	50/2	10.4 \pm 0.7
<i>Pollinating agents</i>			
Bumble bees	83.2 \pm 13.5	50/1	12.0 \pm 1.2
Honey bees	92.1 \pm 9.6	60/1	11.4 \pm 0.9
Mason bees	82.0 \pm 12.0	40/1	10.8 \pm 0.7
Pollen spray	84.6 \pm 12.5	40/1	10.0 \pm 1.3

Only the highest class of fruit size is reported. Class 1: $\varnothing \geq 60$ mm; class 2: $50 \text{ mm} < \varnothing < 59$ mm. No fruits of the diameter class < 45 mm were present. Fruit yield (mean kg per tree \pm sd) of caged trees plots was as follows: 21.4 ± 3.2 (bumble bees); 53.2 ± 8.5 (honey bees); 30.7 ± 4.0 (mason bees); 31.3 ± 3.3 (pollen spray).

size of the colony without modifying its normal foraging activity. When planning our experiments, we had to consider the possibility that, owing to their well documented disorientation in greenhouses or similar caged environments (Giulivo et al., 1970; Douault, 1981; Marletto, 1986), a high number of honey bees could die or at least exhibit a reduced activity. After mason bee pollination, total yield was quite similar to the level reached after pollen spray. Bumble bees showed a fairly good efficiency (Table 3), particularly if one considers the lower number of insects introduced compared to that of honey and mason bees. Fruit quality analysis indicated only one case of significant difference (Student's *t*-test, $P = 0.05$), i.e. between mean fruit weight after honey bee pollination in comparison with mason bees.

4. Discussion

The present work deals with insect controlled pollination of Black Diamond; in Italy, this plum cultivar is strictly self-incompatible (0% fruit set after self-pollination). Our results show that both honey bees and mason bees visit this cv. and that, as demonstrated by the data on yield, their visits are useful for an efficient pollination. Since both insect species were observed while foraging pollenizers, cross-pollinating contacts must have occurred, as previously reported for some Rosaceae (Free, 1962; McGregor, 1976; Torchio et al., 1987; Bosch, 1994a; Benedek, 1996). Regarding bumble bees, they are reported to be fairly good pollinators of plum (Brown, 1951; Calzoni and Speranza, 1996). Therefore, the fact that they apparently did not work in the orchard while they were active on the caged trees, was probably due to not homogeneous quality of colonies (purchased from a commercial firm). Perhaps, the colony introduced in the open field was not at its right developmental age. On the other hand, we can assert that we tried to detect any bumble bee activity in a correct time of counting. In fact, during plum blossoming, weather temperature allows them to forage throughout the day; while they reduce their activity in the early morning and in the evening only in the very warm situations (Prys-Jones and Corbet, 1991).

The weather conditions during blossoming time were never such as to reduce the foraging activity of honey bees, and obviously neither that of mason bees. Therefore, from the data on fruit yield in the open field trials, it is not possible to distinguish between the pollination efficiency of honey bees and mason bees, as both pollinators were always working simultaneously. It has to be noted that they foraged at the same time and on the same tree without disturbing one another.

The lower number of flowers foraged by mason bees compared to honey bees is to be evaluated relatively to the very different number of insects of the two species used in our trials (as previously mentioned, the insect charge was according to McGregor (1976), Bosch (1994b) and Calzoni and Speranza (1996)). In fact, if one considers the number of visits of each insect species as a percentage of the approximate number of individuals of that species introduced in the plantation, the visits of mason bees largely exceeded those of honey bees.

The total number of insect visits throughout blooming, taken as an overall index of foraging activity, is closely connected with fruit yield. Free (1962) also observed

enhanced fruit set on plum trees nearest to honey bee colonies. When considering the quantitative yield of the more visited areas in the open field, it may be noted that it was very similar to the yield obtained after pollen spray.

Under the nets, pollinator visits resulted in satisfactory fruit yield of Black Diamond; in this way, each pollinator was shown to be individually efficient. However, a quantitative comparison between them cannot be done, due to the very different insect charge under the nets.

In conclusion, the pollination technique described by our preliminary trials seems to be quite feasible, easy to manage and suitable for the cv. plum tested. The critical point is to introduce pollinators at their right developmental age and at the right blooming time of the crop. Since some years, bumble bees or mason bees can be purchased also in our region (North Italy). After analysing overall costs per hectare (in Italian Lira, honey bees: 280 000; mason bees: 200 000; bumble bees: 840 000; pollen spray: 450 000), we can conclude that insect controlled pollination—at least by means of honey bees or mason bees—is somewhat cheaper than pollen spray. Furthermore, the use of pollinator insects should also be promoted for its benefits on the agroecosystem as it is necessarily coupled with safe and ecologically sound techniques of pest and weed management. It also helps to increase natural populations of pollinators which have been damaged and drastically reduced after decades of indiscriminate application of chemicals. Finally, we wish to underline that in the future, among the various technologies for a sustainable agriculture, pollinator insects can and should play once again an important role for world food production (Levin and Waller, 1989).

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