Brazilian Agricultural Research Corporation
Embrapa Soybean
Ministry of Agriculture, Livestock and Food Supply

SOYBEAN AND BEES

Decio Luiz Gazzoni

Embrapa
Brasília, DF
2016

Copies of this publication can be adquired at:

Embrapa Soja

Rod. Carlos João Strass, s/n, Acesso Orlando Amaral, Distrito de Warta
Londrina, PR, Brazil
CEP 86001-970
Caixa Postal 231
Phone: +55 (43) 3371 6000
www.embrapa.br
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1st edition

Digitized PDF (2016).

International Cataloging in Publication (CIP) Data
Embrapa Soybean

Gazzoni, Decio Luiz.
Soybean and bees / Decio Luiz Gazzoni. – Brasília, DF : Embrapa, 2016.
147 p. : il. color. ; 21,6 cm x 27,9 cm.

ISBN 978-85-7035-592-8

1. Soja. 2. Polinização. 3. Abelha. I. Embrapa Soja. II. Título.

CDD 633.34

AUTHOR

Decio Luiz Gazzoni

Agronomist, M.Sc. in Entomology,

Researcher at Embrapa Soybean,

Londrina, PR, Brazil

Photo: Paulo Robson de Souza

This book is dedicated to my long time friend **Dr. Arnildo Pott**, a former Embrapa Scientist, presently a Botany Professor at the Universidade Federal do Mato Grosso do Sul. He is a world recognized and widely known botany scholar, whose wisdom, knowledge, scientific contribution, dedication and hardworking attitude I have always admired.

FOREWORD

Brazil, aligned with international efforts, has conducted several studies and activities concerning conservation and sustainable use of pollinators. The loss of primary habitat of these agents, particularly the elimination of native vegetation for multiple uses, ranks as one of the greatest threats to ecosystem services of pollination. In terms of public policies, the Brazilian Forest Law, for instance, can potentially shift the game in favor of the environmental pollination service, by providing shelter and food for pollinators. Embrapa is committed to the study and the quantification of this contribution.

At Embrapa, the growing attention to this issue underscores the need to expand the use of technology, processes and structures to ensure that pollination services are favored. Studies are being developed for continuous monitoring and characterization of pollinators and their contribution to the agricultural production systems used in the country. Special consideration is required to monitor and study the consequences of deforestation and the inappropriate use of pesticides in agriculture, as well as other processes that might pose a disadvantage to the environmental service of pollination.

Furthermore, Embrapa understands that these have great value to agricultural production and the environment. The balance of ecosystems depends on soil fertility, cleanness of water and air, waste decomposition and recycling, as well as the slowdown in the extreme weather events and natural disasters. Therefore, every effort to understand and ensure the integrity of environmental services highly ranks as beneficial to human existence on the planet.

Small-scale farming, conducted in small spaces or in organic production systems, can be performed without using pesticides, under certain circumstances. On the other hand, pesticides constitute an important input for large-scale agriculture, due to operational and economic reasons, although its application should follow the recommendations of Good Agricultural Practices. Therefore, production systems should be improved to favor the action of natural enemies and pollinators.

Consequently, the management of agricultural areas must rely on practices that favor the presence and permanence of beneficial insects in the cropping fields, with an emphasis on the populations of pollinators. The adoption of good farming practices such as integrated pest management often means lower production costs and less risk of disruption of ecosystems. It is also worth mentioning that Embrapa, historically, keeps guidelines to support the continuous development and use of pest management programs, aiming to minimize the pest damage to the crops, the production costs and the negative environmental impact.

In accordance with the historic environmental concern of Embrapa embedded on the technologies developed by its scientists, its research program recently incorporated a research arrangement – a set of correlated projects – called POLIAGRO, which aims to harmonize existing and future production systems comprising the environmental service of pollination. Other arrangements of research projects such as ABELHA and SA (Environmental Services) largely overlaps with the central theme of the POLIAGRO.

The POLIAGRO will integrate existing research networks, or create new ones, involving institutes and universities in Brazil and abroad, under the coordination of Embrapa, generating processes and technologies to help farmers and subsidize public policies, focusing on creating a favorable environment for the pollination service. Besides being a widespread demand in society, the studies to be conducted under POLIAGRO are fully online with concerns of farmers and their organizations, as well as government agencies such as the Brazilian Ministries of Environment, Agriculture, Livestock and Food Supply, and Science and Technology.

The *Soybeans and Bees* book aims to deeply review and discuss the pollination process in soybeans, whose recent increasing productivity, founded on technological innovations, depends on efficient crop management. Soybean is the most important crop in the country, occupying large areas, constituting the highest consumption of pesticides – aspects that can negatively affect the ecosystem service of pollination, which, on the other side, benefits other cultures growing in adjacent agricultural landscape. An aspect that should also be considered is that soybeans is a cleistogamic plant, with low rate of cross pollination, but some studies point out to benefits when bees consistently visit their flowers, an aspect that must be definitively clarified.

The author correctly argues the need to expand the domain of diversity and seasonal abundance of pollinating bees in relation to differential morphological behavior among soybean cultivars, which is crucial to set up a mitigation strategy for the negative impact of pest control actions upon the pollination service.

In my opinion, the effort represented by this book is justified by the economic and environmental importance of soybean production, a crop that has expressively grown over the past three decades and accounts for almost 50% of the grain areas in Brazil. Mainly cultivated in the Central West and South regions, soybean enables a complex consisting of beans, meal and oil, and stands as one of the most traded products in the Brazilian trade balance. The

Ministry of Agriculture, Livestock and Food Supply foresees a 45% increase in Brazilian soybean production by 2019, supported by the expansion of both international demand and domestic consumption, not only for food production but also for industrial purposes, including biodiesel production, a reason for continuously improving the sustainability of soybean production systems.

The effort made by Dr. Decio Luiz Gazzoni in the organization of this book is widely welcomed. With detailed information, presented in an objective, didactic and illustrated way, this publication offers producers, technicians, students, government officials and other interested citizens data and reflections indispensable to expanding the knowledge of the interactions between pollination by bees and soybean cultivation. Performing the harmonization of the ways, means and times needed by the pollination service, it will also be possible to program the application of pesticides in such a way not to harm the pollinators as well as to mitigate present and potential damage to the equilibrium of ecosystems.

Maurício Antônio Lopes
President of Embrapa

PREFACE

Soybeans (*Glycine max* (L.) Merrill) are host of several insect pests along its cycle, since germination to the maturity stage. Exception made for the germination to seedling stage, soybean yield and the quality of seeds and grains are far more affected during the reproductive stage when pods are present, as compared to the vegetative or flowering stages. Pests attacking soybeans during the vegetative stage are chiefly defoliators (lepidopterous and coleopterous), while pod feeders (stinkbugs and pod feeders or pod borers) are more important from pod set to maturity. During the flowering period, before pod set, only defoliator insect pests are considered harmful to soybeans. Nevertheless, this is not true for indeterminate cultivars, because there is an overlapping period of ca. 15-20 days of blooming with pod set and fill and even the beginning of seed fill. In this case, pod feeders, especially stinkbugs, may show up large populations, beyond the action level, requiring a pest control measure. In this moment, care should be taken to avoid or minimize the impact over pollinators.

The soybean plant is hermaphrodite, producing perfect flowers with functional male and female parts. The anthers produce pollen and ovules develop in the ovary in the same flower. If ripe pollen lands on a receptive stigma, the pollen can grow a tube through the style and the pollen nucleus with all of the genetic information can travel through the tube and combine with the egg in the ovule. The ovary protects and nourishes the zygote and supports the development of the embryo, endosperm and seed coat of the seed. In the base of the flower there is a nectary, which produces nectar, a highly nutritive chemical compound that attracts pollinators.

Soybeans are considered autogamous, cleistogamous and self-pollinating plants. When soybean flowers open, they are normally self-pollinated; the stigma of the pistil is completely covered by the anthers of the stamen, making it very difficult for exogenous pollen to reach it. The cross pollination is mentioned in the literature as normally occurring at rates around 2%, being the wind pollination negligible. Cross-pollination on soybeans is mediated by pollinators, normally insects, and especially bees. Bees collect nectar as their major source of energy (carbohydrates) and pollen as the major protein source.

Soybean flower abscission is very high, as the number of harvested pods are in the range of 10-20% of the number of opened flowers. According to the revised literature, abscising flowers were mostly all fertilized and usually contained proembryos that had undergone two or three cell divisions. In this case, apparently there is no interference of insect pests or lack of pollination on the abscission of soybean flowers.

The fertilization of soybean flowers usually occurs one day before or in the very day of flower opening, which theoretically reduces the dependency on pollination by insects, which is largely supported in the literature. Cross-pollination on soybeans usually do not surpass 2%, and is mediated by pollinating insects, normally honeybees. The bees concentrate its foraging on soybeans from 9 am to 3 pm.

Nevertheless, some authors have determined that assisted pollination by honeybees increases the soybean yield, especially due to a higher number of pods and more seeds per pod. Some studies on USA and Brazil found out that, when soybeans are caged with honeybees' colonies inside, yield could increase from 10 to 50%, as compared to caged soybean plots absent of bees. When a soybean yield increase was observed in the presence of bees, the number of filled pods, and the number of seeds per pod were higher than plots grown with absence of bees. There are also references mentioning that no yield difference was found on soybeans cultivated on the presence or absence of bees.

The literature mention a dominance of the honeybee, *Apis mellifera*, foraging on soybean fields, but several native species are also found. There is a clear need to establish the seasonal diversity and abundance of pollinating bees foraging on soybeans, to support the strategy for mitigating the impact of pest control actions upon the pollination service.

A single soybean flower remains open for 1-2 days. The blooming period of soybeans lasts about 15 days for determinate cultivars, and up to 30 days for indeterminate ones. In the case of determinate cultivars, when pods set, the blooming period finishes. In the case of indeterminate cultivars, blooming period extends during pod set and pod development, and might partially overlaps the seed filling stage. As for the present moment, indeterminate varieties are far more cultivated in Brazil, as compared to determinate ones.

These differential blooming behaviors between soybean cultivars is crucial for the compatibility of pollination on soybeans with the control of insect pests. Leaf feeder pests (coleopterons or caterpillars) can attack soybeans along the whole cycle, from early seedling stage up to physiological maturity. Pod feeders (caterpillars or stinkbugs) are considered pests only when pods are larger than 0.5 cm long. On determinate varieties, there is no need to control pod feeder pests during blooming, which is not true for indeterminate ones, given the pest abundance and the level of action recommend by IPM practices. For these cultivars, a period of ca. two weeks is extremely critical, due to the overlapping of flowers and pods, at the same time, on soybean plants.

Honeybee visitation to a flower can be considered a two-stage process. At first involves orientation from a larger distance and then, secondarily, close-range orientation during which the bee alights and probes for nectar. Bees are guided to particular flowers by floral aroma, color, and shape. Floral aroma, color, and shape all therefore appear to influence initial honeybee visitation and provide foraging landmarks, which honeybees utilize to optimize foraging on a specific plant species.

Nectar is a powerful attractant of bees to a given flower. Nectar is a complex of carbohydrates, basically a solution of fructose, glucose, or sucrose in water with minute amounts of many other plant compounds like other carbohydrates, amino acids, proteins, mineral ions, organic acids, vitamins, lipids, antioxidants, glycosides, alkaloids, and flavonoids. Content of carbohydrate in the nectar may vary, from 4 to 60%, depending on species and on environmental conditions, and there is also a variation according to the time of the day, what may determine foraging hours.

The importance of olfaction in recruitment of forager honeybees has been well documented. Honeybees have large numbers of antennal placoid sensilla, which are the principal chemoreceptors for floral aromas. Indeed, it has been suggested that olfaction plays a more important role in forager recruitment than the dance maneuvers observed in colonies. Scent is more important in conditioning honeybees than color, form, or time of day, as honeybee discrimination was greater with a change of scent than with a change of flower pattern or shape.

At first, it is not fairly acceptable that increasing soybean yields would depend on cross-pollination, considering that on cleistogamic plants, when the flower opens it is normally fertilized. Natural cross-pollination in soybeans has been estimated to be low, ranging from about 0.03% to 3.62%. This low cross-pollination would indicate that the entomophilous pollination would represent very low impact on soybean yield. However, there is a controversy on the contribution of pollinators for increasing the yield and seed quality on soybeans. Some authors have found that the presence of pollinators, especially honeybees, in open field environment, or on caged soybeans, lead to the increment of soybean yield. This is still an open issue, because it is not the expected behavior of a cleistogamic, self-pollinating plant, with an average of only 2% cross-pollination. When larger soybeans yields were found, no physiological evidences were stated to support the findings.

In order to minimize the adverse impact upon pollinators of pest control measures that cannot be avoided, studies should be developed to define the most appropriate strategies. Among other should be mentioned that, during this period, it is paramount to strictly observe the IPM recommendations, controlling pests when absolutely necessary to avoid yield or seed quality reduction. Minimum rates of insecticides with less impact on pollinators should be used. Pesticide application should preferentially be performed during periods of the day when bee populations on soybean fields are lower, or even absent, especially early on the morning, late on the afternoon or at night. Additionally, components of the production systems deterrents to pollinators should be adequate, while those favoring the natural pollination services must be reinforced.

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SOYBEAN CYCLE

Soybean (*Glycine max* (L.) Merrill, family Fabaceae, subfamily Faboideae) hosts several insect pests along its cycle, since germination to the maturity stage. Exception made for the germination to seedling stage, soybean yield and the quality of seeds and grains are far more affected during the portion of the reproductive stage when pods are found on the plants, as compared to the vegetative or flowering stages. Pests attacking soybeans during the vegetative stage (Table 1 and Figure 1) are chiefly defoliators (lepidopterous and coleopterous). Scientists have definitively demonstrated that soybeans can withstand severe defoliation rates, even up to 100%, prior to blooming, without loss of yield or quality. This ability demonstrates a sound resilience to these biotic stresses, given abiotic environmental (temperature and water) stresses are not present, and adequate cultural practices (soil management; plant nutrition; weeds, nematodes and diseases control) are observed (GAZZONI et al., 1978).

Table 1. Soybean growth stages.

a. Vegetative stages		
Stage	**Stage title**	**Description**
VE	Emergence	Cotyledons above the soil surface
V1	First node	Fully developed leaves at unifoliolate nodes
V2	Second node	Fully developed trifoliolate leaves at node above the unifoliolate nodes
V3	Third node	Three nodes on the main stem with fully developed leaves beginning with the unifoliolate nodes
Vn	n_{th} node	"n" nodes on the main stem with fully developed leaves beginning with the unifoliolate nodes.

b. Reproductive stages

Stage	Stage title	Description
R1	Beginning bloom	One open flower at any node on the main stem
R2	Full bloom	Open flower at one of the two uppermost nodes on the main stem with a fully developed leaf
R3	Beginning pod	Pod 5 mm (3/16 inch) long at one of the four uppermost nodes on the main stem with a fully developed leaf
R4	Full pod	Pod 2 cm (3/4 inch) long at one of the four uppermost nodes on the main stem with a fully developed leaf
R5	Beginning seed	Seed 3mm (1/8 inch) long in a pod at one of the four uppermost nodes on the main stem with a fully developed leaf
R6	Full seed	Pod containing green seed that fills the pod cavity at one of the four uppermost nodes on the main stem with a fully developed leaf
R7	Beginning maturity	One normal pod on the main stem that has reached its mature pod color
R8	Full maturity	Ninety-five percent of the pods have reached their mature pod color

Source: Fehr and Caviness, 1971; 1977.

Photos: Decio Luiz Gazzoni

VE. (Germination and cotyledon). V1. Unifoliolate. V3. Two trifoliolate open.

V4. Three trifoliolate open. V6. Five trifoliolate open.

Figure 1. Soybean vegetative stages.

Soybeans become more susceptible to insect pests attack during the reproductive stage, especially after blooming (pods are present on the plants). During blooming, the plant still withstands large defoliation rates (up to 50%), being more susceptible to foliar area losses from pod set to physiological maturity (R7) (GAZZONI and MINOR, 1979; GAZZONI and MOSCARDI, 1998).

Nevertheless, insect attacking pods and seeds are far more noxious to the soybean plant than defoliators are. Their importance is limited to the R3-R7 soybean growth stages (Figures 2 and 3). Their attack might result in loss of entire pods and seeds, or reduced seed weight. The recovery ability of soybean plant decreases as pods increase in size, or according the development of seeds (FRASER at al, 1982).

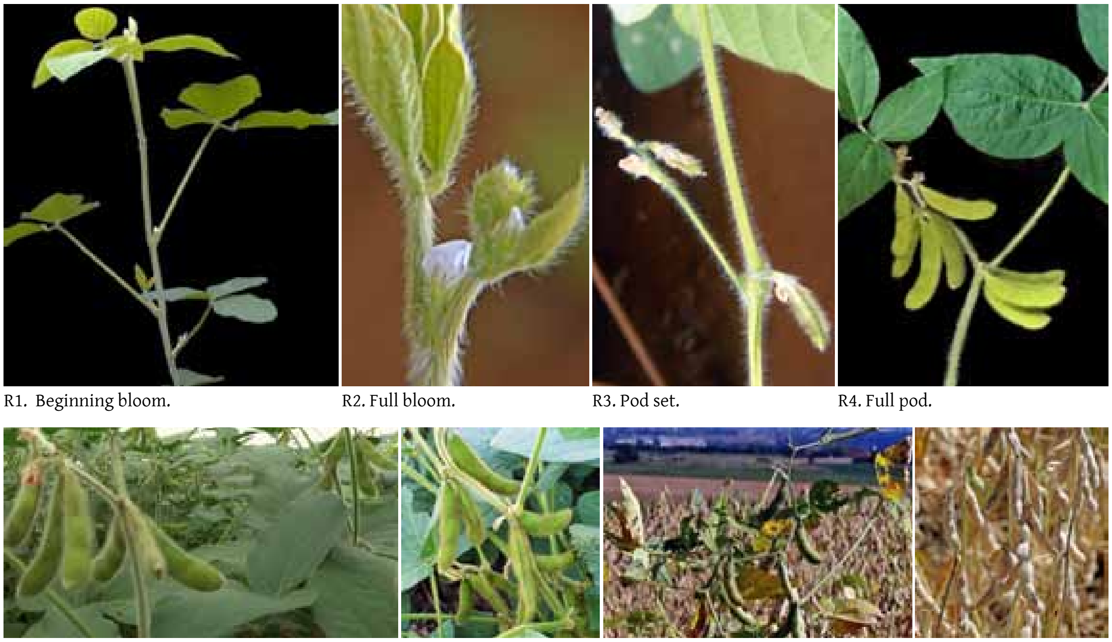
The attack of stinkbugs to soybean seeds may lead to irreversible damage when attacking bugs affect the hypocotyl-radicle axis, which prevents the seed or affects seedling emergence (CORSO, 1977).

Sometimes, bug attacks can trigger physiological unbalance, and affected plants do not adequately complete its cycle, slowing the maturity process, causing leaf retention and troubling mechanical harvesting (SILVA and RUEDELL, 1983). Bugs are also responsible for disease transmission, since the location where mouthparts penetrate the seeds allows intrusion of pathogenic organisms in seeds, like the fungus *Nematospora coryli* and also species of bacteria. Besides yield reduction, severe attacks of stinkbugs result in reduced oil content and increased grain protein content (CORSO and PORTO, 1978).

It is important to mention that stinkbugs may colonize soybean plants in various stages of development. However, the ability to cause damage is restricted to its attack directly to pods and seeds, thus no noxious effects were observed prior to pod set and close to harvest. It is frequently observed even considerable populations of these insects prior to the blooming stage, increasing progressively in the reproductive phase, with an exponential growth, even accelerated at the end of the crop cycle, especially in the case of medium and late maturity cultivars, which remain longer in the field. This population increase is not only due to insects coming from eggs laid on the same field where they are observed. In most cases, population growth is due to the intense migration of adult insects from recently harvested crops, in search of better shelter, feeding and reproduction conditions.

Soybean can stand a given level of stinkbug population without reducing its yield or seed quality. A pioneer and probably the most important and conclusive study on this area was conducted by Villas Boas et al. (1990), who studied for seven consecutive years the effects of different populations of bugs on soybean yield and seed quality. The authors found that on plots where the action level for controlling the pest were populations up to four bugs/m of soybean row, there was no statistical difference in the productivity and quality of seeds, as compared to plots with no stinkbugs (zero population). When the action level was set over four bugs/m, the yield consistently decreased, being also affected the seed viability and vigor.

Photos: Decio Luiz Gazzoni



R1. Beginning bloom. R2. Full bloom. R3. Pod set. R4. Full pod.

R5. Beginning seed fill. R6. Full seed (green bean). R7. Physiological maturity. R8. Harvest maturity.

Figure 2. Soybean reproductive stages.

Photos: Decio Luiz Gazzoni

R3. Lenght= 8 mm. R4. Lenght= 20 mm. R5. Lenght= 40 mm.

R6. Lenght= 45 mm. R7. Lenght= 45 mm. R8. Lenght= 45 mm.

Figure 3. Pods and seeds size (mm) from stages R3 to R8 of soybean development.

Luckily for the growers - and for the pollinators - during the blooming period, when bees use to forage on soybeans, pod and seed feeder pests usually are not important – because pods and seeds are not yet present on the plants. However, while this is quite true for determinate soybean cultivars, the situation is more complex in indeterminate ones, due to the overlapping of blooming, pod set and pod fill stages, which are quite distinct along the cycle on determinate varieties. While blooming is over at the end of R3 stage for determinate cultivars, flowers may still be observed on the plants up to the R6 stage on indeterminate cultivars.

In order to avoid negative impacts of the control of soybean pests on bees and other pollinators, it is crucial to understand the reproductive anatomy and physiology of the soybean plant, as well as the synchrony of pest attack and its damages according to the stage of soybean development. Concepts like pest monitoring, levels of damage and action as well as diversification of pest control strategies, play an important role to reach this target.

In addition, it is important to review details of the anatomy and physiology of soybean reproduction in order to understand the unique resilience of soybeans to insect pest damage, not only during the vegetative but also during the reproductive stage, especially while pods are not present on the plants. This specificity will help to design Pest Management practices, fine-tuning the strategy in order to consider the minimum impact on pollinators visiting soybeans.