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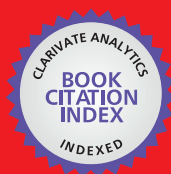
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Intensity of Powdery Mildew in Soybean Under Changes of Temperature and Leaf Wetness

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1. Introduction

Soybean *Glycine max* L. Merr. is cultivated in several tropical and subtropical regions of the world. United States (USA) and Brazil are the world's largest producers and exporters of oilseed (Agrianual, 2008; Miyasaka & Medina, 1981).

Despite the high production and export of Brazilian soybeans, many factors have affected the quality or quantity of production of that crop, causing reduction in financial returns per unit area, such as disease epidemics. Among the diseases, powdery mildew, whose etiologic agent is *Microspheera diffusa* Cke. & Pk., suddenly began to cause significant damage in soybean, despite having a broad host range and have been reported in Brazil, Canada, Republic of China, India, Puerto Rico, South Africa, United States (Sinclair, 1999), Germany, Argentina and Bolivia (Sartorato & Yorinori, 2001).

According to Yorinori & Hiromoto (1998), crops widely affected by the disease, had estimated reductions between 30 and 40% of yield, in the same order of magnitude as those reported abroad by Dunleavy (1978) and Philips (1984). The susceptibility of cultivars and the influence of climate favored epidemics with high rates of disease progress, in successive years in Brazil. Considering the lack of resistance of most of the cultivars, chemical control is required, especially in the south and the high plains of the savannah biome (Sartorato & Yorinori, 2001). In 1996/97, epidemics of powdery mildew in soybean in a great extent of Brazil, from the Central West region to the Rio Grande do Sul state, resulted in average losses of 15 and 20% in susceptible cultivars, with extremes ranging from 50 to 60% (Yorinori & Hiromoto, 1998 ; Seganfredo & Silva, 1999).

M. diffusa is distinguished from *M. polygoni* by presenting cleistothecium with appendages forked at its end (Sartorato & Yorinori, 2001; Grau, 1975). The fungus is an obligate parasite that develops throughout the soybean shoot, including leaves, stems, petioles and pods. Symptoms can range from chlorosis, green islands, rusty spots, defoliation or severe combination of these symptoms, depending on the reaction of cultivars. Chlorotic spots and necrosis on the leaf veins indicate a hypersensitivity reaction. However, the most obvious is the very structure and powdery white fungus on the surface of infected parts (Yorinori, 1982; Yorinori, 1986, Tanaka et al., 1993; Yorinori et al., 1993; Sinclair, 1999; Sartorato &

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Yorinori, 2001). In general, the lower leaves of young plants are more susceptible than the upper leaves (Mignucci & Lim, 1980).

In relation to physiological changes in the host, Mignucci & Boyer (1979) studied the inhibition of photosynthesis and transpiration of soybean infected with powdery mildew and found lower photosynthesis and transpiration with increased infection. With 82% of leaf area infected, more than half of the leaf photosynthetic activity had been lost and transpiration dropped to 36% compared to control, considering the direct result of the change in metabolic activity induced by the pathogen. Because infection occurs primarily in the lower leaves and poorly lit, it is unlikely that the reduction in rates of photosynthesis and leaf transpiration resulted in great reduction in soybean yield, however, favorable climatic conditions may enabled the infection of upper leaves leading to high losses (Mignucci & Boyer, 1979; Sartorato & Yorinori, 2001).

Susceptibility of cultivars and influence of the climate has caused outbreaks of powdery mildew in successive years in Brazil. The lack of resistance in most cultivars have required chemical control mainly in the south and the high plateaus of the savannahs. In the U.S.A., powdery mildew caused economic damage reached in the 70's and early 80's. Since then, the use of resistant cultivars has dispensed chemical control (Sartorato & Yorinori, 2001).

Reactions of different soybean varieties to powdery mildew and the effect of environmental variables in the progress of the disease have been reported (Arny et al. 1975; Buzzell et al. 1975; Degree & Laurence, 1975, Johnson & Phillips, 1961; Mignucci 1977; Mignucci & Boyer, 1979; Mignucci & Lim, 1980; Lohnes & Bernard, 1992; Lohnes & Nickell, 1994). According to Bedendo (1995), in Brazil, powdery mildew may occur in the humid and cold climates, but are favored by hot dry conditions (20-25 °C). According to the author, conidia do not germinate when is present a film of water on the leaf surface, however, relative humidity near 95% is required for germination.

Mignucci et al. (1977) reported temperatures of 18 °C as favorable to the development of powdery mildew on susceptible cultivars and at temperatures of 30 °C disease progress was inhibited. Degree & Laurence (1975) also observed lower disease severity at 30 °C. According to Sartorato & Yorinori (2001) the information about the effects of relative humidity, leaf wetness, rainfall, solar radiation or other environmental factors in the progress of powdery mildew in soybeans was not precise.

Therefore, the intensity of powdery mildew of soybean under different temperatures and periods of leaf wetness on the cultivars conquista and suprema was evaluated.

2. Material and methods

Seeds of soybean cultivar conquista (MG/BR 46) and suprema were sown in pots containing 5 kg of soil mixture, sand and organic matter (manure) in the proportion 2:1:0.5 in a green house. Thinning was performed 15 days after planting, leaving two plants per pot, forming the experimental unit. The plants were kept in a green house until the V3 stage, according to the soybean phenological scale proposed by Ritchie et al. (1982). During the same period, inoculation of *M. diffusa* was done stirring soybean plants on healthy plants which were then randomly placed next to diseased plants (Demski & Phillips, 1974). According to Grau (1975), because of the ease with which conidia are disseminated, it becomes hard to test inoculation of *M. diffusa* with different isolates without contamination.

The plants were transferred to growth chambers and arranged in randomized blocks, factorial 4 x 5 with three replicates, considering four air temperatures (15, 20, 25 and 30

degrees C) and five leaf wetness periods (0, 6, 12, 18 and 24 hours). For the different periods of leaf wetness, recently sprayed plants were kept in a moist chamber with transparent plastic bags, during the period used for each treatment. In the treatment of 0 h of wetness, the plants were taken without a moist chamber for the growth chambers. Irrigation was performed by spraying water directly on the stem of the plants.

There were four incidence and severity assessments every five days after the beginning of the experiment. The severity was assessed on all central leaflet of each plant with trifoliate leaves at 9, 11, 13 and 15 days after inoculation, using the grading scale published Sartorato & Yorinori (2001), adopting grade 1 = 1% of affected leaf area; grade 2 = 5%, grade 3 = 10%, grade 4 = 25%, grade 5 = 50%, grade 6 = 100%.

The intensity data were integrated using the area under incidence progress curve over time, according to Campbell & Madden (1990).

$$AUDPCS = \sum_i^{n-1} \left(\frac{ys_i + ys_{i+1}}{2} \right) (t_{i+1} - t_i) \quad (1)$$

$$AUDPCI = \sum_i^{n-1} \left(\frac{yi_i + yi_{i+1}}{2} \right) (t_{i+1} - t_i) \quad (2)$$

Where:

AUDPCI was the area under the progress curve of powdery mildew incidence; AUDPCS was the area under the progress curve of powdery mildew severity; ys and yi were the disease severity and incidence over time i and $i+1$, respectively; t was the time in days and n was the number of evaluations along the time.

Plants with chlorosis, green islands, rusty stains, and combination of these symptoms were considered infected by powdery mildew (Figure 1).

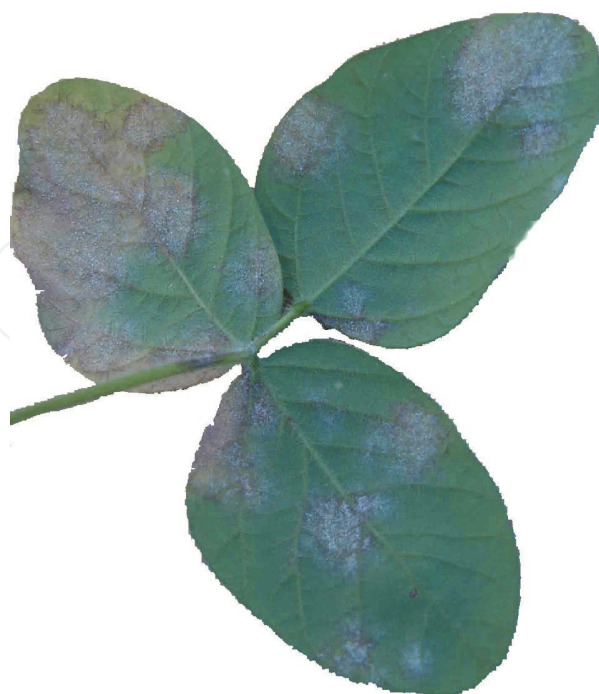


Fig. 1. Evaluated signals of powdery mildew in soybean plants.

The significant variables in the F test by the variance analysis of AUDPCS were subjected to regression analysis, linear and nonlinear adjustment models (Leite & Amorim, 2002, Reis et al., 2004). In the case of significant interaction, the combined effect of temperature and leaf wetness duration in disease intensity was modeled (Reis et al., 2004).

3. Results and discussion

Symptoms of powdery mildew evaluated at 9, 11, 13 and 15 days after inoculation, were characterized by chlorosis, green islands, rusty stains, and combination of these symptoms in the cultivars suprema and conquista. However, the most striking characteristic evaluated was the presence of the fungus, and powdery white structure on the surface of infected leaves. This symptomatology was consistent with those described by Sartorato & Yorinori (2001), Tanaka et al. (1993), Yorinori et al. (1993) and Yorinori & Hiromoto (1998). According to Sartorato & Yorinori (2001) and Yorinori et al. (1998), may also be variations in the symptoms of powdery mildew due to climatic variations, genetic variability between populations of *M. diffusa*, genetic resistance of cultivars, stage of plant development and adopted agronomic practices. Tanaka et al. (1993) studying the occurrence of powdery mildew (*M. diffusa*) in a collection of 27 soybean genotypes, in a green house, observed differences of severity symptoms presented by By Hampton cultivars (more susceptible), followed by IAC-Foscarin 31 and IAC-Santa Maria 702, respectively. According to Lohnes & Bernard (1992), Lohnes & Nickell (1994) and Mignucci & Lim (1980), the differing responses of powdery mildew in soybeans are consequences of three alleles at locus Rmd: Rmd-c (resistant), Rmd (resistance in adult plants) and rmd (susceptibility) and according to Dunleavy (1978) and Phillips (1984), these differences may be evidenced by a 35% loss of productivity in soybean cultivars susceptible to powdery mildew in the field.

A significant interaction in the F test between temperature and leaf wetness was observed for the AUDPCS in conquista cultivar ($P = 0.0242$) and the isolated effect of temperature in conquista ($P < 0.0001$) and suprema ($P < 0.0001$). Thus, models were adjusted using non-linear regression to describe the monocyclic process of the epidemic based on the dependent variables. With regard to temperature, a greater amount of disease was observed at temperatures around 23 °C for the conquista and 24 °C for the suprema cultivar. Temperatures above 15 °C and 30 °C were not favorable to the development of powdery mildew in both cultivars (Figures 2, 3 and 4).

Likewise Leath & Carroll (1982) in a study on powdery mildew of soybean cultivars, evaluating 38 cultivars, observed greater susceptibility of cultivars Ware, Falcon, AP350, V76-438, Emerald, AgDSR232, AgDSR532, Md71-583 as well as smaller and larger disease progress (*M. diffusa*) in Georgetown, under temperature of 29.6 °C and 23.2 °C, respectively. However, Mignucci et al. (1977) found at temperatures of 18 °C in a green house, the greater progress of powdery mildew in Flambeau, Norchief, Chippewa 64, Corsoy, Harosoy 63, Wells cultivars,, grown in the USA and Puerto Rico. Seedlings were subjected to temperatures of 18, 24 and 30 °C per 14 hours, with alternating 10 hour temperature of 20 °C, to simulate day and night temperatures. In further studies, the same authors, after inoculation of *M. diffusa*, in cultivar Harosoy, in a green house, diseased plants were kept in a growth chamber under daytime temperature of 26 ± 2 °C and night 21 ± 2 °C (Mignucci & Chamberlain, 1978) and at 25 ± 0.25 °C (Mignucci & Boyer, 1979). However, both Mignucci et al. (1977), Mignucci (1989) and Leath & Carroll (1982) agreed that temperatures around 30 °C were not favorable to disease progress, similar to that observed in the cultivars

evaluated in this study. In another pathosystem, powdery mildew of grape (*Uncinula necator* (Schw.) Burr.), The optimum temperature for growth of the fungus was 25 °C, while in the temperature between 21 and 30 °C there was germination of spores and increased sporulation. At temperatures above 33 °C occurred death of spores and colonies (Thomas et al., 1994; Reis, 2004).

$$\text{AUDPCS} = (54.5327) \exp(-(2.86394) \left(\frac{(T - (23.1927))}{(9.03343)} \right)^2 + ((M - (8.24455)) / (14.5439))^2)) \quad R^2 = 0.85 *$$

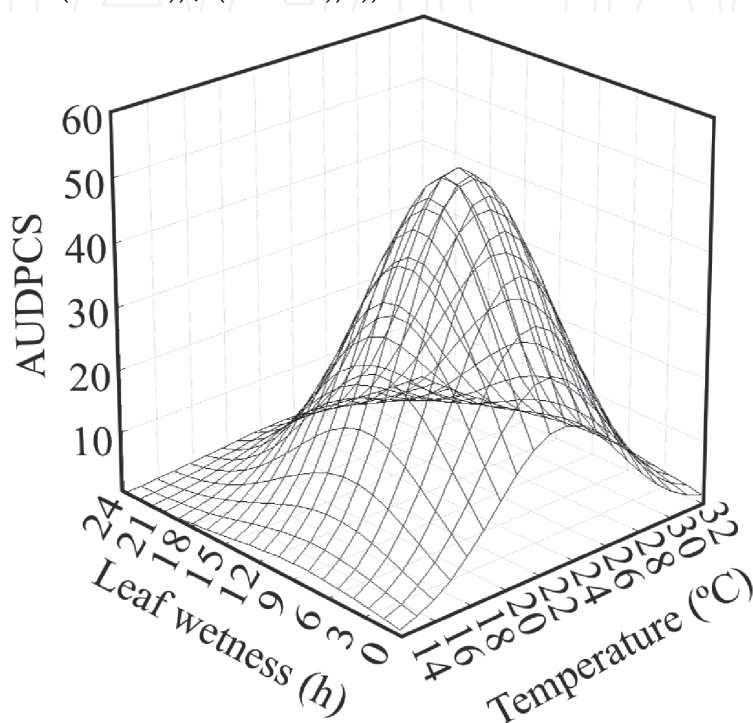


Fig. 2. Nonlinear regression of the progress curve of disease severity (AUDPCS) of powdery mildew of soybean in conquista cultivar according to the interaction between temperature and leaf wetness.

With regard to leaf wetness in conquista cultivar, there were signs of the disease from 0 to 8 hours of leaf wetness, with growth up values of AUDPCS until 8 hours, with the maximum of temperature of 23 °C, indicating the need of water for germination of spores and fungus infection. From that point, higher values of leaf wetness reduced the AUDPCS (Figure 2). With respect to the isolated effects of leaf wetness periods in suprema cultivar, the maximum point of leaf wetness in the AUDPCI occurred in the period of 12.9 hours, with significant reduction near 0 and 24 hours (Figure 5). There is little information in the literature about the effects of leaf wetness on powdery mildew in soybeans (Sartorato & Yorinori, 2001), however, according to Bedendo (1995), this disease can occur in humid regions, but is favored by dry environments. Mignucci (1989) reported that the low relative humidity is highly favorable precisely described the development of powdery mildew in soybeans, though not presented values to describe precisely. Similarly, Brodie & Neufeld (1942) studying the development of conidial structures of *Erysiphe polygoni* DC., found germination in relative humidity ranging from 0-100%, while Mattiazzi (2003) studying the effect of mildew on the soybean production, observed greater progress at a relative

humidity of 80%. Thus, the relative humidity of the growth chamber, with an average of 50%, may have given the conidial germination, even in treatments with no leaf wetness.

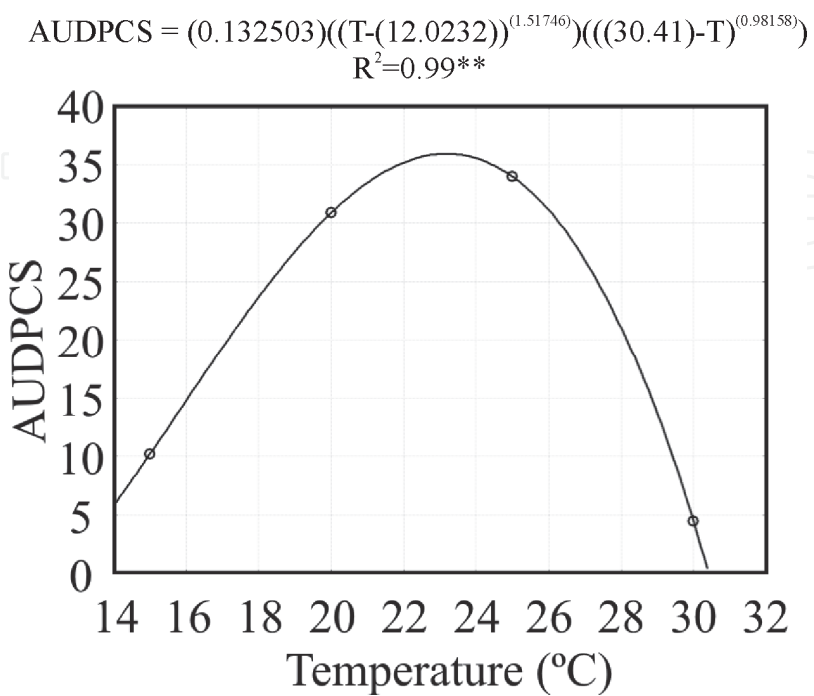


Fig. 3. Nonlinear regression of the progress curve of disease severity (AUDPCS) of powdery mildew of soybean in conquista cultivar according to the isolated effect of temperature.

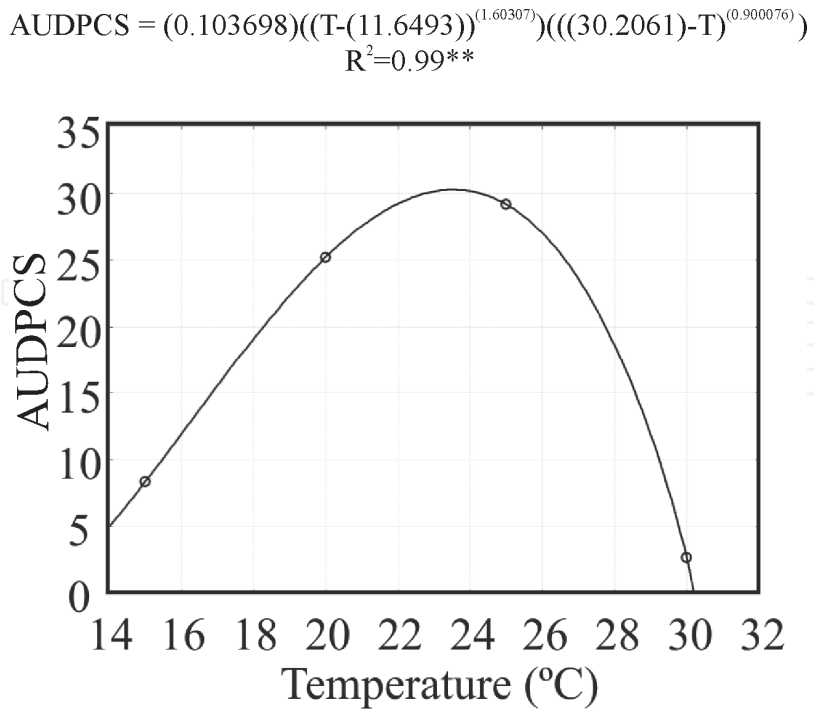


Fig. 4. Nonlinear regression of the progress curve of disease severity (AUDPCS) of powdery mildew of soybean in suprema cultivar according to the isolated effect of temperature.

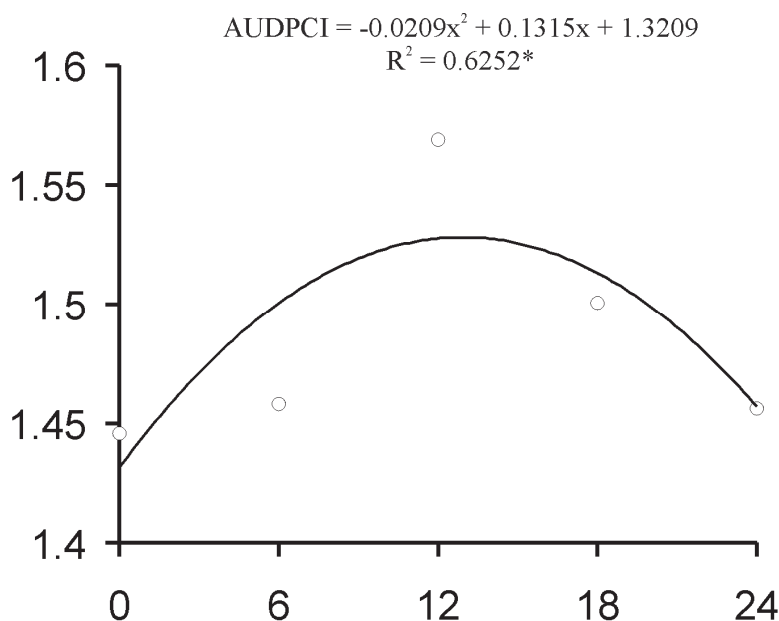


Fig. 5. Linear regression with polynomial quadratic fit of the progress curve of disease incidence (AUDPCI) of powdery mildew of soybean in suprema cultivar (Y axis) according to the isolated effect of leaf wetness (X axis).

Therefore, the results on the effect of leaf wetness in the progress of powdery mildew (*M. diffusa*) are contradictory and studies on the interaction effect between temperature and duration of leaf wetness on disease progression had not yet been assessed. The results presented potential use in prospective studies on the effects of weather on the progress of powdery mildew of soybean cultivars in Brazil.

4. Conclusion

The progress of the severity of powdery mildew (AUDPCS) in suprema and conquista cultivars was favored by air temperatures around 23 °C and 24 °C, respectively.

Leaf wetness of 8h and air temperature of 23 °C provide the maximum progress of disease severity in conquista cultivar.

Temperatures above 30 °C and 15 °C reduced the intensity of the disease.

5. Summary

In this study the effects of temperature and leaf wetness period on the intensity progress of powdery mildew in soybean conquista and suprema cultivars were evaluated. Plants at the V3 stage were inoculated in greenhouse. Subsequently, the plants were conditioned in growth chambers at temperatures of 15, 20, 25 and 30°C and leaf wetness periods of 0, 6, 12, 18 and 24 hours. Severity data was integrated in time by the disease progress curve for incidence (AUDPCI) and severity (AUDPCS). Non-linear regression models were adjusted for the disease severity and a polynomial fit was adjusted for disease incidence data. Temperatures near 23 °C and 24 °C favored the powdery mildew intensity progress

(AUDPCS) in Conquista and Suprema cultivars, respectively. Leaf wetness period of 8 h allowed the maximum progress of the disease in conquista at temperatures of 23 °C. Temperatures near 30 °C and 15 °C reduced powdery mildew intensity. The maximum point of leaf wetness in the AUDPCI occurred in the period of 12.9 hours, with significant reduction near 0 and 24 hours

6. References

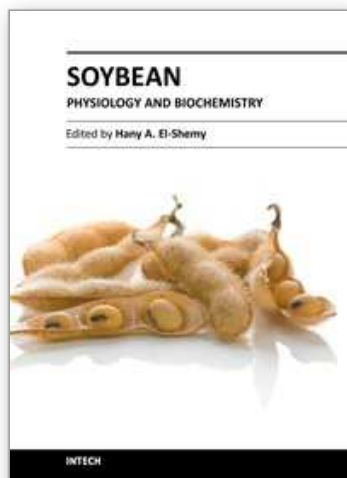
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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soyfoods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

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