UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF CANOPY ARCHITECTURE AND SEASONAL VARIATIONS ON SEVERAL SEED QUALITY ATTRIBUTES IN SOYBEAN (GLYCINE MAX L. MERR.)

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By

ERENSO DEGU GUTEMA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

To:
My beloved wife Yeshihareg Kebebew,
My children Lidia, Henock and Girum
and
to the memory of my parents.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Adam Puteh, PhD

Faculty : Agriculture

Producing high quality soybean seed in the hot humid tropics is no easy task. During seed production, several environmental factors and plant morphological characteristics can exert their influences on seed quality. A study was undertaken at Universiti Putra Malaysia to study the effect of canopy architecture and seasonal variations on several seed quality attributes in soybean (*Glycine max* L. Merr.). Four soybean cultivars namely, Palmetto, AGS190, Deing and Cikurai were grown in the field for four seasons during 2003 and 2004. Four levels of defoliation treatments [0% defoliation (±170.89µmol $m^{-2}s^{-1}$ light intensity), 25% defoliation (±324.33µmol $m^{-2}s^{-1}$ light intensity), 50% defoliation (±473.01µmol $m^{-2}s^{-1}$ light intensity) and 75% defoliation (±642.84µmol $m^{-2}s^{-1}$ light intensity], were imposed at the pod initiation stage (R3). Weather factors such as light intensity, canopy, air and soil temperatures, canopy and air relative humidity, soil moisture and leaf area index were recorded at seven-day intervals starting from the imposition of defoliation until plants reached physiological maturity (R7). Seeds harvested at harvest maturity (R8) were used to determine seed yield,

viability, vigour, 100-seed weight and for *Phomopsis* bioassay. *Phomopsis* sp. seed infection was predicted using weather factors and leaf area index. Scanning electron microscopy (SEM) was used to study the progression and colonization of *Phomopsis* sp. on the stem, pod and seed starting from R3 until R8.

Defoliation treatments were found to have inconsistent effects on seed yield (kg ha^{-1}) and pods per plant for AGS190, Deing and Palmetto. Pod number of Palmetto was affected during season III only whereas the pod number for Cikurai was significantly affected for all seasons. However, defoliation treatments affected 100-seed weight for all cultivars except for AGS190 (season I), Deing (season IV) and Cikurai (season III).

From the combined analysis of data over the four seasons, defoliation improved percent seed germination from 6.8 to 13.2%. Increasing the level of defoliation resulted in increasing percent germination and 3-day seedling height and reduced *Phomopsis* incidence for all cultivars. The highest germination was recorded during season II which coincided with the least level of *Phomopsis* sp. seed infection. Moreover, this disease was influenced by seasonal variations. The disease incidence was high during seasons III (51.3%) and IV (49.5%) characterized by high rainfall during seed development and maturation as compared to seasons I and II which encountered low rainfall situations (33.3% and 32.5%, respectively). AGS190, a large seeded cultivar, was severely affected by the seed-borne disease (51.5% infection) whereas Deing, a small seeded cultivar, was the least affected (34.7%).

Defoliation treatments increased light intensity within the plant canopy for all cultivars studied. Light intensity and canopy temperature revealed negative correlation with percentage *Phomopsis* incidence indicating that high light intensity and temperature inside plant canopy reduced *Phomopsis* sp. seed infection. On the contrary, positive relationship was observed between canopy and air relative humidity, soil moisture and leaf area index with percentage *Phomopsis* incidence. From the stepwise multiple regression analysis, *Phomopsis* sp. seed infection can be predicted by leaf area index, soil moisture and canopy relative humidity; while its reduction can be predicted by increased light intensity and canopy temperature during seed development and maturation.

Scanning electron microscopy revealed that fungi progression and colonization started at different growth stages for different plant parts. Stems were infected during the early reproductive stage (R3) whereas pods became noticeably infected during the full seed stage (R6) and seeds were the last to be infected. The fungi were internally- borne within the infected seeds. Fungal hyphae were observed externally on the surface of the plant parts studied and internally both in the pod and in all the three layers of the seed coat: palisade cell, hourglass cell and parenchyma cell layers. Fungi colonization was highest at the late plant growth stages of R7 and R8.

A progressive increase in pod and seed infection was detected during subsequent growth stages between R6 and R8. From the pod, the pathogen can infect and colonize the seed. The SEM results suggested that the reproductive growth period of R6 to R7 was more critical with respect to *Phomopsis* sp. seed infection than earlier reproductive growth periods, since more severe colonization of pods and seeds took place at the later stages of plant growth. SEM revealed that stem infection allowed buildup of inoculum for subsequent infection of the pod, whereas pod infection was necessary for further infection of seeds. Since pod infection is the prerequisite for seed infection, this study suggests that fungicide would be best applied between R4 and R5 before the seed-borne fungi reach the seed during R6 and the subsequent growth stages.

Prediction model based on four seasons' data accurately described the relationship between the environmental conditions and leaf area index during seed development and maturation and the levels of seed infection by *Phomopsis* sp. Moreover, the model fitted well with the field and laboratory data collected. However, this model needs to be tested at multilocational trials for validity.

The results of the present study have shown that plant canopy modification through defoliation appears to improve quality of seeds produced under wet and warm tropical environments. In addition, the study also suggested that growing of soybean cultivars with open canopies and having low leaf area index, coupled with rain-free harvesting seasons can result in the production of high quality seeds. Although the prediction model so developed in this study needs to be tested for validity at different locations and variable environments, it has the potential to be used as a practical tool in plant disease forecasting programs.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN RUPABENTUK KANOPI DAN PERBEZAAN MUSIM KEATAS CIRI KUALITI BIJIBENIH KACANG SOYA (*Glycine max* L. Merr.)

Oleh

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Menghasilkan biji benih kacang soya di kawasan tropika yang panas dan lembab bukannya satu kerja mudah. Semasa pengeluaran biji benih, beberapa faktor persekitaran dan ciri morfologi tumbuhan boleh mempengaruhi kualiti biji benih. Satu kajian telah dijalankan di Universiti Putra Malaysia untuk mengkaji kesan rekabentuk kanopi dan perbezaan musim ke atas beberapa ciri kualiti biji benih kacang soya (*Glycine max* L. Merr.). Empat kultivar kacang soya, iaitu, Palmetto, AGS190, Deing dan Cikurai telah ditanam di ladang untuk empat musim dalam tahun 2003 dan 2004. Empat tahap rawatan defoliasi [0% defoliasi (±170.89µmol $m^{-2}s^{-1}$ intensiti cahaya), 25% defoliasi (±324.33µmol $m^{-2}s^{-1}$ intensiti cahaya), 50% defoliasi (±473.01µmol $m^{-2}s^{-1}$ intensiti cahaya) dan 75% defoliasi (±642.84µmol $m^{-2}s^{-1}$ intensiti cahaya] telah dilakukan di peringkat permulaan pembentukan lenggai (R3). Faktor cuaca seperti intensiti cahaya, suhu silara, udara dan tanah, kelembapan relatif silara dan udara, kelembapan tanah dan indeks keluasan daun telah direkodkan selang tujuh hari bermula dari tarikh rawatan defoliasi dikenakan sehinggalah tanaman mencapai kematangan

fisiologi (R7). Biji benih yang dituai semasa kematangan ladang (R8) telah digunakan untuk penentuan hasil, kecergasan, berat 100 biji dan viabiliti biji benih, dan untuk bioassay *Phomopsis*. Jangkitan *Phomopsis* sp. pada biji benih telah diramal menggunakan faktor cuaca dan indeks keluasan daun. Imbasan mikroskop elektron telah digunakan untuk mengkaji kemajuan dan pengklonian *Phomopsis* sp. pada batang, lenggai dan biji benih bermula dari R3 sehingga R8.

Rawatan defoliasi menunjukkan kesan tidak konsistan keatas hasil bijibenih (kg ha^{-1}) dan jumlah lenggai per pokok bagi AGS190, Deing dan Palmetto. Defoliasi memberi kesan keatas lenggai per pokok bagi Palmetto pada musim III sahaja, manakala kesannya terhadap kultivar Cikurai adalah untuk semua musim penanaman. Rawatan defoliasi didapati mempengaruhi berat 100-bijibenih untuk semua kultivar kecuali bagi AGS190 (musim I). Deing (musim IV) dan Cikurai (musim III).

Dari analisis gabungan data untuk ke empat-empat musim, defoliasi meningkatkan percambahan biji benih dari 6.8 ke 13.2%. Meningkatkan tahap defoliasi menghasilkan peningkatan peratus percambahan dan ketinggian anak benih 3-hari dan mengurangkan kehadiran *Phomopsis* untuk semua kultivar. Percambahan paling tinggi telah direkodkan pada musim II dan jangkitan *Phomopsis* terhadap biji benih juga adalah paling rendah pada musim itu. Lebih-lebih lagi, penyakit ini adalah dipengaruhi oleh perbezaan musim. Kehadiran penyakit adalah tinggi pada musim III (51.3%) dan IV (49.5%) bilamana hujan banyak semasa pembentukan dan kematangan biji benih. AGS190, satu kultivar berbiji benih besar, telah dijangkiti secara serius oleh penyakit bawaan biji benih (51.5%) manakala Deing, satu kultivar berbiji benih kecil, adalah paling sedikit dijangkiti (34.7%).

Rawatan defoliasi meningkatkan intensiti cahaya di dalam silara tumbuhan untuk semua kultivar yang dikaji. Intensiti cahaya dan suhu silara menunjukaan korelasi negatif dengan peratus kehadiran *Phomopsis*, sekaligus menunjukkan bahawa intensiti cahaya dan suhu yang tinggi di dalam silara mengurangkan jangkitan *Phomopsis* terhadap biji benih. Di sebaliknya, korelasi positif telah diperhatikan di antara kelembapan relatif silara dan udara, kelembapan tanah dan indeks keluasan daun dengan peratus kehadiran *Phomopsis*. Dari analisis regresi berganda, jangkitan *Phomopsis* sp. terhadap biji benih boleh diramalkan oleh indeks keluasan daun, kelembapan tanah dan kelembapan relatif silara; manakala pengurangannya boleh diramalkan dengan pengurangan intensiti cahaya dan suhu silara semasa pembentukan dan kematangan biji benih.

Imbasan mikroskop elektron menunjukkan bahawa kemajuan dan pengklonian kulat bermula pada peringkat pertumbuhan berbeza bagi bahagian-bahagian tumbuhan yang berbeza. Batang dijangkiti pada peringkat reproduktif awal (R3) manakala lenggai kelihatan jelas dijangkiti di peringkat pertumbuhan biji benih penuh (R6) dan biji benih pula adalah yang paling akhir dijangkiti. Kulat tersebut didapati berada di dalam biji benih yang dijangkiti. Beberapa hypha diperhatikan berada di luar permukaan beberapa bahagian tumbuhan yang dikaji dan berada di dalam lenggai dan ketiga-tiga lapisan kulit biji: sel palisad, sel "hourglass" dan sel parenkima. Pengklonian kulat adalah tertinggi pada peringkat akhir pertumbuhan, R7 dan R8.

Jangkitan progresif terhadap biji benih dan lenggai telah dikesan semasa peringkat pertumbuhan di antara R6 dan R8. Dari lenggai, patogen boleh menjangkiti dan mengkloni biji benih. Hasil imbasan mikroskop elektron menunjukkan bahawa peringkat reproduktif R6 ke R7 adalah lebih kritikal berhubung dengan jangkitan biji benih oleh *Phomopsis/ Colletotrichum* sp. dari peringkat reproduktif yang lebih awal, kerana pengklonian yang teruk terhadap lenggai dan biji benih berlaku di peringkat akhir pertumbuhan. Imbasan mikroskop elektron juga membuktikan bahawa jangkitan batang membenarkan pengumpulan inokulum yang menyebabkan jangkitan lenggai kemudiannya, manakala jangkitan lenggai pula adalah satu keperluan untuk menyebabkan jangkitan pada biji benih. Oleh kerana jangkitan lenggai merupakan keperluan untuk jangkitan biji benih, maka kajian ini menyarankan agar aplikasi racun kulat adalah sangat sesuai dilakukan di antara R4 dan R5 sebelum kulat bawaan biji benih sampai ke biji benih di peringkat R6 atau peringkat pertumbuhan selanjutnya.

Model ramalan berdasarkan data dari empat musim penanaman dengan tepat menerangkan hubungan di antara keadaan persekitaran dan indeks keluasan daun semasa pembentukan dan kematangan biji benih dengan tahap jangkitan *Phomopsis* sp. terhadap biji benih. Lagi pun, model ini adalah padan dengan data yang dikumpul dari lapangan dan juga makmal. Walau bagaimana pun, model ini perlu diuji di kajian multilokasi untuk menentukan kesahihannya.

Hasil kajian ini telah menunjukkan bahawa modifikasi rekabentuk silara melalui defoliasi boleh meningkatkan kualiti biji benih yang dihasilkan di persekitan tropika yang lembab dan panas. Tambahan lagi, kajian ini juga menunjukkan bahawa penghasilan biji benih kacang soya oleh kultivar yang mempunyai silara terbuka dan mempunyai indeks keluasan daun yang rendah, disekalikan dengan musim penuaian tanpa hujan boleh menghasilkan biji benih berkualiti tinggi. Walaupun model ramalan yang dihasilkan dalam kajian ini masih perlu ditentukan kesahihannya melalui ujian di pelbagai lokasi dan persekitaran berbeza, ia berpotensi untuk digunakan sebagai satu alat yang praktikal dalam program peramalan penyakit tumbuhan.

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ANOVA	Analysis of variance
AOSA	Association of seed analysts
d	Day (s)
DPC	*Diaporthe/Phomopsis* complex
EC	Electrical conductivity of seed leachates
ISTA	International Seed Testing Association
LAI	Leaf area index
NPK	N= nitrogen, P = phosphorus and K= potassium
PDA	Potato dextrose agar
Phom	Phomopsis
PM	Physiological maturity
RH	Relative humidity
SAS	Statistical Application System
SEM	Scanning electron microscopy
SG	Standard germination
sp.	Species
UPM	Universiti Putra Malaysia