Kasetsart J. (Nat. Sci.) 29 : 252-265 (1995)

Effects of Host Growth Stage, Inoculation Time, Inoculation Method, Fungicides and Host Hybrid Line on Alternaria Blight Development of Sunflower.

Sutruedee Prathuangwong, Samang Wongkoay[1]

ABSTRACT

The severity of *Alternaria* spp. and their effects on yield depending on host growth stage, inoculation time, inoculation method, fungicides, and host hybrid line were evaluated. Disease development was greatest when sunflowers were inoculated at R1 growth stage, during 09.00-11.00 am, by the method of placing 20-30 grains of sorghum grain culture of mixed inoculum of *A. helianthi*, *A. zinniae*, and *A. alternata*. A foliar spray of iprodione and mancozeb plus with Triton CS-7 reduced the severity of Alternaria blight and increased yield of sunflower which was significant difference from non-sprayed plants. The 3 hybrid lines, Hysun 33, KU line, and Synthetic #3 tested for reaction to *Alternaria* spp. in the field experiment, were susceptible with no differences on disease severity, 100 seed weight and yield among them. These studies revealed that sunflower genotypes can be screened in the field for resistance to infection by *Alternaria* spp. using the techniques described herewith.

Key word : Alternaria blight, *A. helianthi*, *A. zinniae*, *A. alternata*, sunflower (*Helianthus annuus*)

INTRODUCTION

Infection in sunflower (*Helianthus annuus*) by *Alternaria helianthi*, *A. zinniae*, and *A. Alternata* has become a common disease (stem, head, and leaf blight) in Thailand (Prathuangwong *et al*, 1989; Prathuangwong *et al.*, 1991) and around the world (Allen *et al.*, 1981; Herr and Lipps, 1982; Morris *et al.*, 1983). Although little information is available on the economic importance of Alternaria blight, its common occurrence and yield loss studies conducted in some growing areas indicate that it is a potentially serious disease with losses as great as 60 % after field inoculation (Allen *et al.*, 1981: Carson, 1985a; Carson, 1985b). However, no information have been available on Alternaria blight based on the reliable data of the studies under local conditions. Therefore, research on this disease is essential to establish the appropriate technology for sunflower production in various environmental conditions.

There is need for a reliable inoculation technique that will result in a sufficiently high level of infection by *Alternaria* spp. in sunflower. Screening of lines or cultivars for resistance to these

[1] Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

pathogens under field condition is still questionable because the levels of infection depends on several factors which may or may not be suitable for infection and disease development. Environmental conditions, plant age, time of inoculation, method of inoculation and chemical substances are more or less related to the plant infection. Consequently, a reliable screening procedure is desirable to facilitate repeatable evaluation of disease resistance in sunflower cultivars or lines to *Alternaria* spp. The data obtained will not only improve the technique to induce the plant infection more easily and rapidly resulting in increased infection within limited time but will also give the guidelines for direct control of the disease effectively.

At present, Thailand has introduced several sunflower cultivars/lines into the country, attempt are made to breed cultivars/lines for better yield and adaptability to different agroclimatic conditions. Some of the parents involved in the breeding programs may be susceptible to Alternaria blight. If no selection pressure for disease resistance is applied in the segregating populations obtained from crossing, it is possible that the new cultivars/lines developed might be susceptible. Such an erosion of resistance can nullify the advantages of higher yields expected from such crosses.

Two fungicides have been tested to control Alternaria blight of sunflower are iprodione and mancozeb. These fungicides reduced disease severity and provided higher yield than nontreated plants (Prathuangwong *et al.*, 1991). This study, therefore, was conducted to determine and reconfirm the efficacy of the two foliar fungicides on Alternaria blight development and yield of sunflower.

The purpose of this investigation included the determination of the effect of sunflower growth stage, time of inoculation, and method of inoculation on Alternaria blight to develop a method for screening sunflower cultivars/lines for resistance to *Alternaria* spp. Disease assessment key developed to make quantitative disease assessment and reaction of some hybrid lines representative of sunflower germplasm used in Thailand to Alterneria blight were also studied.

MATERIALS AND METHODS

The 3 sunflower hybrid lines, Hysun 33, KU line, and Synthetic #3 were used in all experiments. Plants were grown in 4.0 m long and 2.25 m apart, spacing 0.40 x 0.75 m, path 2 m end of each plot with 4 rows per plot and 10 plants per row. Treatments were arranged in factorial experiment in a completely random design with 4 factors and 9 levels. These were factor I : 2 sunflower growth stages (initial inoculation at the V6 and R1 growth stage); factor II : 2 periods of time at inoculation during the day (at 09.00 - 11.00 am and 19.00 - 21.00 pm); factor III : 2 methods of inoculation (placing of 20-30 grains of sorghum grain culture and spraying of spore suspension); factor IV: 3 lines of sunflowers (Hysun 33, KU line, and Synthetic #3) with 24 treatment combinations. Control of each treatment was sprayed at 14 days interval from V6 to R8 growth stages with a mixture of iprodione (50 WP), 25 g/20 L water and mancozeb (80 WP), 25 g/20L water; plus with Triton CS-7, 5 ml/20L water with hollow cone nozzle of automized pressure sprayer. Each plant was sprayed until cover all leaves. Laboratory and field studies were done at Department of Plant Pathology, Kasetsart University, Bangkhen Campus; and National Maize and Sorghum Research Center, Pakchong, Nakorn Ratchasima, respecively. All treatments were replicated three times and had been investigated for 2 growing seasons. The data obtained were the average of late rainy season and dry season during 1989-1990.

Inoculum. Single conidial isolate of *A. helianthi*, *A. zinniae* and *A. alternata* were used for mixed inoculum. Each isolate obtained from sun-

flower growing at 10 farms around the country. Isolates were chosen on the basis of their virulence in preliminary tests on hybrid Hysun 33.

Inoculum for field tests was produced in 2 methods of spore suspension and grain culture. For spore suspension, *A. helianthi* and *A. alternata* were grown on fresh potato carrot agar (PCA: 20 g potato, 20 g carrot, 12 g agar in 1,000 ml distrilled water); *A. zinniae* on sunflower leaf extract agar (100 g sunflower leaf, 12 g agar in 100 ml distilled water) under continous NUV light in 9-cm-diameter petriplates. The media used would induce sporulation better than mycelial growth (Prathuangwong *et al.*, 1991). Inoculum was prepared by scarping and mixing of 3-4 week-old flooded culture of each species into sterile distilled water and adjusted spore concentration to be 1.5 x 10^3 spores/ml with hemacytometer determination. The suspension, however, was plus with Triton CS-7 (5 ml/20 L) before used.

For grain culture inoculum, sorghum seed medium was prepared as described previously (Prathuangwong *et al*, 1991). One to two-week old culture disk (0.5 cm in diameter) of each pathogen was inoculated into sorghum seed medium and incubated under NUV light. Each day, culture was shaked to provide good growth of the pathogen until eventually distributed or it was incubated at least 8 days.

Inoculation techniques. The two techniques compared in this study were spraying of spore suspension (spray inoculation), and placing of 20-30 sorghum grain culture on the plants (place inoculation). Spore suspension was poured into automized pressure sprayer (0.7 kg/cm^2) and about 8-10 ml of inoculum was sprayed on the top leaves of each plant. The plants were predisposed for infection by water spray one hour before inoculation and humid condition was maintained for 1-2 hours after inoculation. Another technique used in the comparison was place inoculation by placing 20-30 grains of sorghum grain culture of the mixed inoculum into the leaf whole of plant tested. Humid condition was maintained as spray inoculation.

Effect of host growth stage on disease expression. Two different host growth stages V6 and R1 were compared. Inoculations were done by either technique of spray or place inoculation on plant wholes when they reached to the growth stage of V6 and R1. The experiment was made as a factorial with 3 replicates arranged in CRD as described previously.

Effect of time at inoculation during the day. The inoculation of mixed inoculum on 3 lines of sunflower Hysun 33, KU line, and Synthetic #3 were done at 2 different times during the day between 09.00-11.00 am and 19.00-21.00 pm when the plants reached to V6 and R1 growth stage. A factorial in CRD with 3 replicates was arranged in all experiments.

Reaction of sunflower lines to Alternaria blight. Three sunflower hybrid lines, Hysun 33, KU line, and Synthetic #3 were evaluated in field experiments for resistance to Alternaria blight during the 1989 and 1990 growing season on the National Maize and Sorghum Research Center, Pakchong, Nakorn Ratchasima. The hybrid lines were selected as being representative either of commercial hybrid (Hysun 33) or germplasm used in breeding program of Kasetsart University (KU line), and of Khon Kaen University (Synthetic #3) . Individual plots consisted of 4 rows with 4 m long and 2.25 m apart. Approximately, thirty seeds per row were hand-planted, stands were thinned, and only one plant per hole was left. *H. annuus* accessions were inoculated at growth stage V6 comparing with the inoculating at R1. Inoculations were done by either spray or place techniques described previously.

Disease and yield assessments. In all experiments, infected leaf areas were measured by the stencil card of 12 x 21 cm with 9 holes (1.5 cm

in diameter of each hole) placing on diseased leaf, the number of holes with lesions and the total number of holes on leaf were counted. The disease severity was calculated as the equation of disease severity in percent as number of holes with lesion/ total number of holes on leaf (9 holes) x 100. This assessment was modified from Prathuangwong and Preecha's method (1990). Disease severity was evaluated at R7 growth stage of sunflower plants. A numerical rating system was used to record the severity of infected leaves as 0 = no infection; 1 = 1-5 % infection of leaf area; 2 = 6-25 % infection; 3 = 26-45 % infection; 4 = 46-65 % infection; 5 = 66-85 % infection, and 6 = 86-100 % infection of leaf areas (or dead). The percent leaf area infected was determined by visually examining each leaf of individual plant. A disease reaction for each sunflower lines was evaluated by averaging the disease severity ratings derived for individual plant. In this study, disease reactions 0 = immune; 1 = highly resistance; 2 = resistance, 3 = moderately resistance; 4 = moderately susceptible; 5 = susceptible; and 6 = highly susceptible. However, number of lesions per stem, lesions per flower and lesions per branch of control plants which were chemical and non- chemical spray were also determined added with percent infection of leaf area as for disease severity evaluation. Disease severity of control plants in this investigation however, was a natural infection and/or natural dispersal.

Yields were harvested from 20 plants per experiment plot. The 100-seed weight and yield were measured after harvest when seed has 13 % moisture content.

Statistical analysis. The effects of treatments were assessed by analysis of variance (ANOVA). The interactions among factors tested were determined. ANOVAs were combined over growing seasons because of no differences in the number of factors and the success of inoculations. Different means in each data recorded were analyzed using Duncan's multiple range test.

RESULTS

Every sunflower hybrid line tested in the investigation developed recognizable lesions. The first symptom of infection on leaves was the appearance of small, water soaked area on the 5th or 6th day after inoculation. These areas became necrotic lesions or dark flecks later. The lesions on mature leaves expanded and increased in number during the following week until they were in a range of 1.5-3.2 mm in diameter. These lesions were usually surrounded by a distinct yellow halo and may coalesce and enlarge into blackened area. Other plant parts included leaf vein, stem, branch, petal, head and receptacle were also damaged resulting in different shapes of dark flecks or streaks (Figure 1).

Effect of host growth stage on disease expression. Disease severity or infected leaf area development was more severe in every sunflower line inoculated at R growth stage than plants inoculated at V growth stage but there was no statistical difference among them (Table 1). Sunflower hybrid line that showed greatest disease severity was KU line followed in sequent order by synthetic #3 and Hysun 33 giving percentage of infected leaf of 85.48, 82.38 and 81.16, respectively. However, KU line showed lowest of disease severity for plants inoculated at V growth stage followed by Hysun 33 and Synthetic #3 which was 78.29, 80.85 and 81.46 % respectively (Table 1). When number lesions per stem was evaluated for disease severity, plants inoculated at R1 growth stage was higher in number of infection than V6 growth stage with significant difference in statistic at 0.05 level (Figure 2). The highest infection was found in Synthetic #3 with 4.67 lesions/stem followed by Hysun 33, and KU line with 4.42 and 4.00 lesions/ stem, respectively (Figure 2).

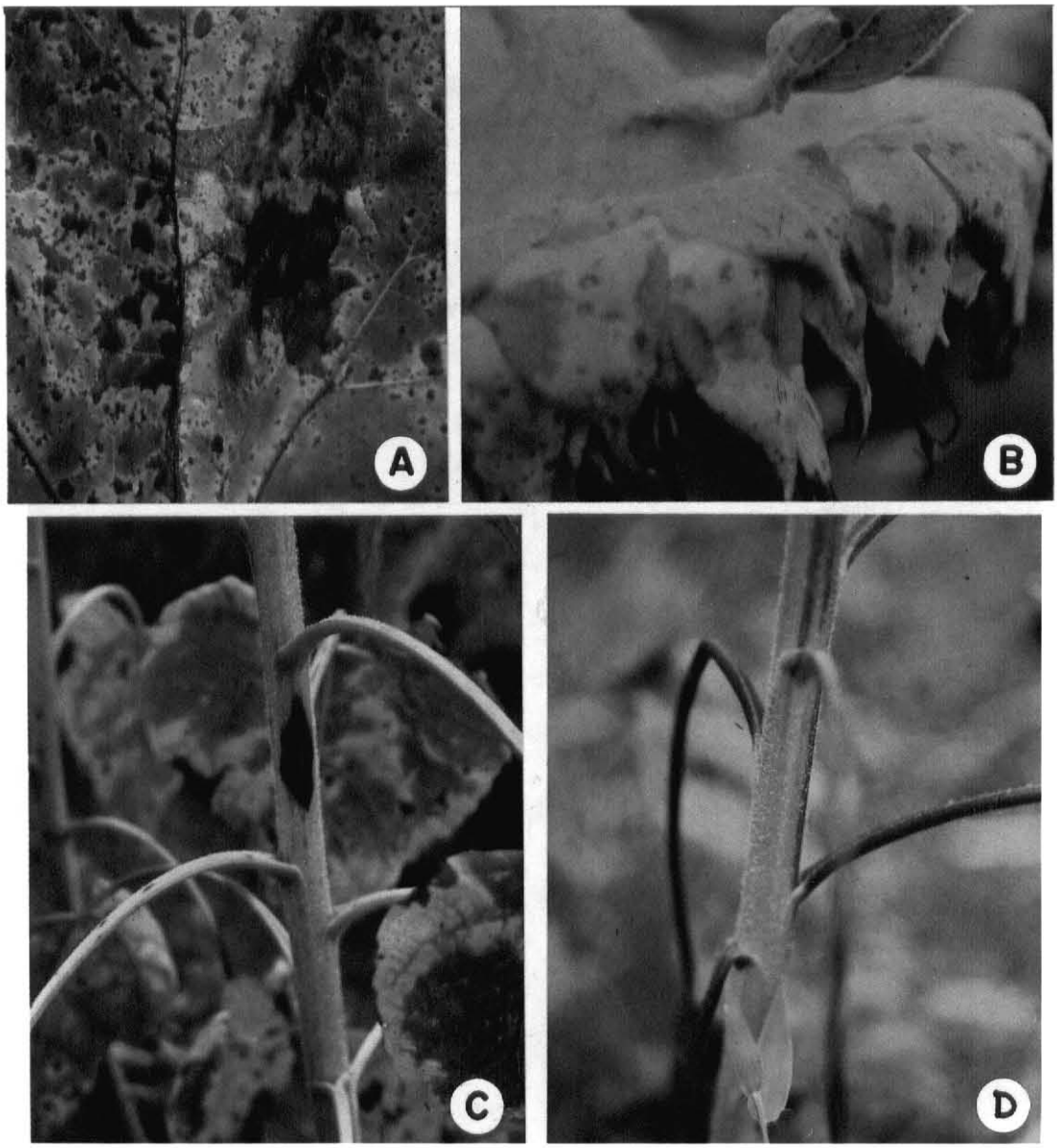

Figure 1 Symptoms of sunflower leaf and stem blight affected with *Alternaria* spp. A, the irregular dark brown spots on the leaf which may coalesce and enlarge into blackened area. B, the pathogen infected the entire head and bract showing sunken dark-brown lesions. C, elliptic shaped lesion on stem and scattered over the stalk. D, black lesions on node, and petioles resulted in drooping and dying of leaves.

Table 1 Effects of host growth stage, time of inoculation, method of inoculation, and host hybrid lines on disease severity in 3 sunflower lines inoculated with *Alternaria helianthi, A. zinniae*, and *A. alternata* as mixed inoculum.

Factors	Treatments	Disease severity[1/] (%)			Means	
		Sunflower hybrid lines				
		Hysun 33	KU line	Synthetics #3		
Host growth stage	V6	80.85 a	78.29 a	81.46 a	80.20	2.61ns
	R1	81.16 a	85.48 a	82.38 a	83.01	
Time of inoculation	09.00-11.00	82.60 a	84.08 a	83.84 a	83.51	4.81*
	19.00-21.00	79.41 b	79.70 b	79.99 b	79.70	
Method of inoculation	Spray	79.55 b	81.23 a	80.08 a	80.28	1.03ns
	Placed	82.46 a	82.54 a	83.76 a	82.92	
	Control[2/]	57.50 d	67.40 c	63.80 c	62.9	
	Means	77.65	79.91	79.33		
Disease reaction[3/]		5	5	5		

ns = Non-significance, * = significant different (P = 0.05) as determined by DMRT.

1/ = Disease severity was measured on % infected leaves by the modification of Prathuangwong and Preecha's method (1990).

2/ = Combinend average disease severity of 60 plants which was natural infection and was foliar spray with fungicides.

3/ = Combined average disease reaction of 60 plants was evaluated as
0 = immune (no infection);
1 = highly resistance (1-5 % infection of leaf area);
2 = resistance (5-25 % infection);
3 = moderately resistance (26-45 %);
4 = moderately susceptible (46-65 % infection);
5 = susceptible (66-85 % infection);
6 = highly susceptible (86-100 % infection).

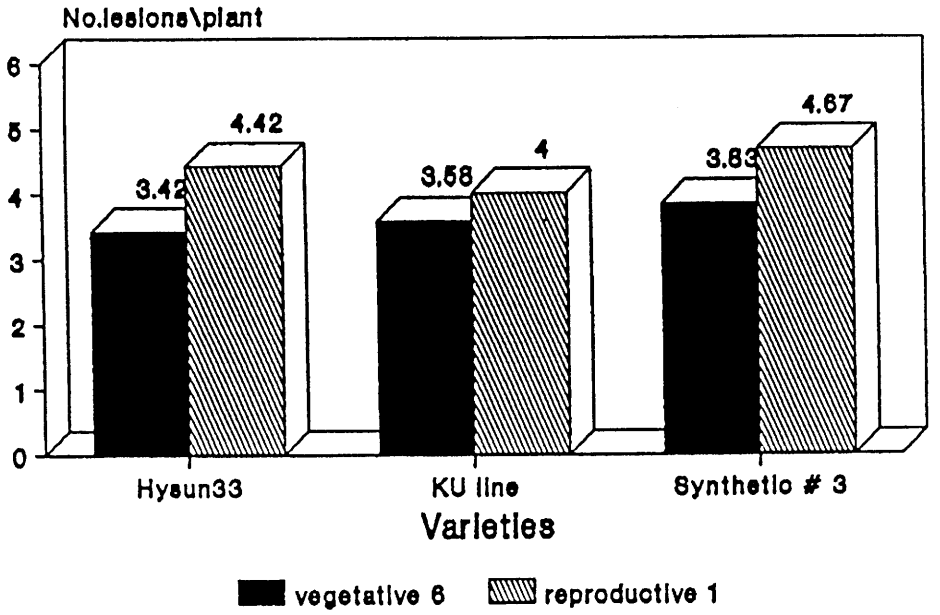

Figure 2 Effect of host growth stage on number lesions per stem in 3 sunflower lines inoculated with mixed inoculum of *Alternaria helianthi, A. zinniae.* and *A. alternata.*

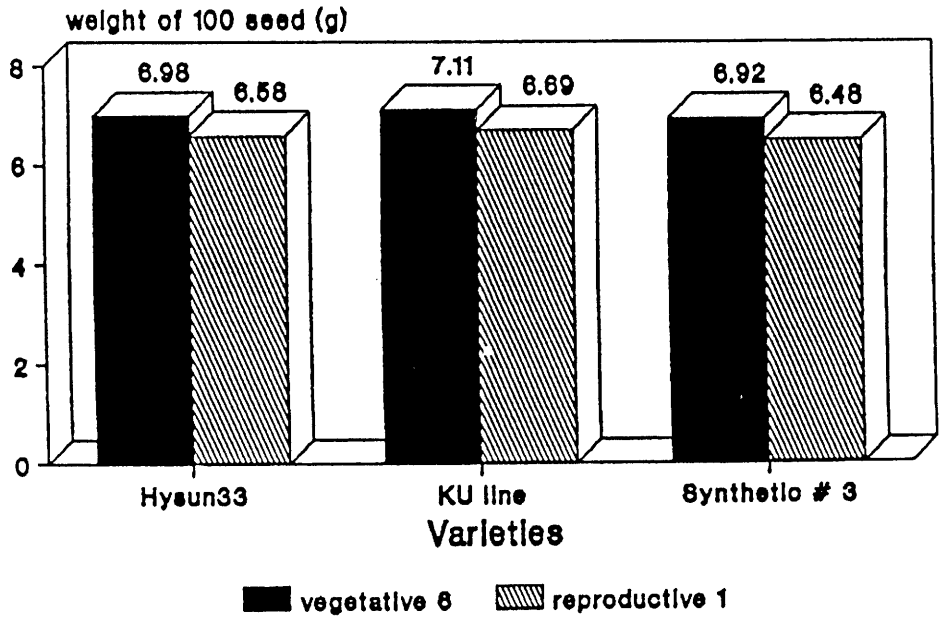

Figure 3 Effect of host growth stage on 100-seed weight of 3 sunflower lines inoculated with *Alterneria helianthi, A. zinniae,* and *A. alternata.*

The 100-seed weight of every sunflower line was also smallest when plants were inoculated at R growth stage compared to V growth stage with giving 6.48, 6.58 and 6.69 g for Synthetic #3, Hysun 33, and KU line, respectively (Figure 3). Significant difference (P = 0.01) in 100-seed weight of plants inoculated at two growth stage was found with average of 6.58 and 7.00 g for R and V stage of inoculation. One hundred-seed weight of plants inoculated at V was 7.11, 6.98 and 6.92 for KU line, Hysun 33, and Synthetic #3, respectively (Figure 3).

No statistical difference in seed yield of plant inoculated at different growth stages was found, but yield of plant inoculated at R was smaller than V in every sunflower lines. That was yield of plant inoculated at R/V equaled 215.33/ 244.17, 216.67/219.99, and 207.58/211.33 kg/rai for Hysun 33, KU line, and Synthetic #3, respectively. The result revealed that in this treatment Hysun 33 gave the highest yield in kg/rai of 244.17 followed by KU line and Synthetic #3 of 219.00 and 211.33 kg/rai, respectively.

Effect of time at inoculation during the day. Alternaria blight development was affected differently among hybrids at difference times of inoculation. Inoculation at 9.00-11.00 am was higher in leaf area of infection than at 19.00-21.00 pm with significant difference (P = 0.05). Disease severity in different lines ranged from 79.41 to 84.08 % with the rating of disease reaction equaled 5 or susceptible level (Table 1). Seed size and seed yield among the 3 sunflower lines inoculated at different times were varied from 6.66 (for Hysun 33 and Synthetic #3) to 6.93 g/100 seeds (for KU line) and from 208.50 (for Synthetic #3), to 220.33 kg/rai (for Hysun 33), respectively. Average seed size and seed yield of plants inoculated at 09.00-11.00 am were 0.78 and 8.06 % smaller than plants inoculated at 19.00-21.00 pm, respectively.

Effect of inoculation technique on disease expression. The placed inoculation technique consistently resulted in higher leaf infection of 82.92 % in average than the spray inoculation technique at 80.28 % in average with no statistical difference among the sunflower hybrids tested except in Hysun 33 (Table 1). The spray inoculation method resulted in significantly lower incidence of leaf infection in Hysun 33 than the place inoculation method. In an average, however, the two methods did not cause differential responses of disease reaction among those hybrids (Table 1). One hundred-seed weight of Hysun 33, KU line, and Synthetic #3 inoculated by spray/place technique were 6.96/6.61, 6.76/7.04 and 6.71/6.68 g, respectively and their yields were 239.08/220.42, 216.17/219.50, and 202.25/216.67 kg/rai, respectively. In this treatment, Hysun 33 gave the highest yield of 239.08 kg/rai while KU line gave the lowest yield of 202.25 kg/rai with no statistical different among 3 hybrids tested.

Reaction of sunflower lines to Alternaria blight. The reactions of 3 sunflower hybrid lines to Alternaria blight inoculated with *Alternaria helianthi, A. zinniae*, and *A. alternata* in the field experiments are presented in Table 1 and Table 2. All plants tested were susceptible with rating disease reaction of level 5. Those lines however, varied in disease severity with mean severities ranged from 77.65 % in Hysun 33 to 79.91 % in KU line (Table 1) and 72.25 % in Hysun 33 to 81.92 % in Synthetic #3 (Table 2) and significant differences (P = 0.05) were detectable. Combined over Table 1 and 2 mean Alternaria blight ratings ranged from 74.95 % (Hysun 33) to 80.87 % (KU line). These lines however, produced lesion types, sizes and shapes similar to each other which were dark brown margins and brown to gray centers, frequently surrounded by either small or enlarge chlorotic halo (Figure 1). The result indicated that all 3 lines of *Helianthus annuus* included Hysun 33, KU line and Synthetic #3 were susceptible to mixed

Table 2 Interaction effects of sunflower line x method of inoculation x host growth stage on 100-seed weight; and line x host growth stage x time of inoculation x method of inoculation on disease severity in 3 sunflower lines inoculated with mixed inoculum of *Alternaria helianthi, A. zinniae,* and *A. alternata.*

Sunflower lines	100-seed weight (g)		Sunflower growth stage	Time of inoculation	Disease severity (%)		Means[1/] disease severity (%)	Disease[2/] reaction	Infected[3/] leaf area of control (%)
	Inoculation technique				Inoculation technique				
	Spray	Placed			Spray	Placed			
Hysun 33	6.95 a	7.02 a	V6	09.00-11.00	84.27 a	83.30 a	72.25	5	57.50
				19.00-21.00	75.10 ab	80.70 a			
	6.97 a	6.20 b	R1	09.00-11.00	73.80 b	89.03 a			
				19.00-21.00	85.03 a	76.77 ab			
KU line	7.13 a	7.08 a	V6	09.00-11.00	78.50 a	82.77 a	81.89	5	67.40
				19.00-21.00	78.63 a	73.27 b			
	6.38 ab	7.00 a	R1	09.00-11.00	89.13 a	85.90 a			
				19.00-21.00	83.90 a	83.00 a			
Synthetic #3	7.10 a	6.73 a	V6	09.00-11.00	78.10 a	84.47 a	81.92	5	63.80
				19.00-21.00	81.73 a	81.53 a			
	6.32 ab	6.63 a	R1	09.00-11.00	77.73 ab	95.07 a			
				19.00-21.00	82.73 a	73.97 b			

Means in each column followed by the same letter are not significantly different according to DMRT.

1/ Combined average disease severity of 240 sunflower plants.

2/ Disease reaction was evaluated as 0 = immune (no infection of leaf area;
1 = highly resistance (1-5 % infection of leaf area); 2 = resistance (6-25 % infection);
3 = moderately resistance (26-45 % infection); 4 = moderately susceptible (46-65 % infection);
5 = susceptible (66-85 % infection); 6 = highly susceptible (68-100 % infection of leaf area).

3/ Control plant was natural infection and was sprayed with iprodione plus mancozeb and Triton CS-7.

inoculum of *Alternaria* spp. eventhough only the low inoculum density of 1.5 x 10^3spores/ml was used in the investigation.

A significant (P = 0.05) hybrid line x host growth stage x time of inoculation x method of inoculation, and hybrid line x method of inoculation x host growth stage interaction was detected in the combined analyses for Alternaria blight indicating disease severity and 100-seed weight are depended on several related factors (Table 2). Within hybrids, disease severity was greatest for Hysun 33 inoculated at R during 19.00-21.00 pm by place inoculation (89.03 %), for KU line inoculated at R host growth stage during 09.00-11.00 am, by the method of spray inoculation (89.13 %), and disease severity was greatest for Synthetic #3 inoculated at R host growth stage during 09.00-11.00 am by the method of place inoculation (95.07 %). The 100-seed weight was affected similarly (Table 2). Inoculation at R host growth stage reduced the seed size lower than V in all sunflower lines and it was varied upon the method of inoculation x lines. In Hysun 33, weight of 100 seeds was lowest when plant was inoculated by the method of place inoculation (6.20 g). In KU line. 100-seed weight was smallest for plants inoculated at R1 host growth stage by the method of spray inoculation (6.38 g) and for Synthetic #3 inoculated at R host growth stage by the method of spray inoculation giving the lowest 100-seed weight of 6.32 g (Table 2).

Effect of foliar fungicides on Alternaria blight. Foliar fungicides with the combination of iprodione, mancozeb, and Trition CS-7 were effective against Alternaria blight in all trials and reduced disease severity in 100 % of the trials. Disease severity of nontreated control (naturally infected plant) was as high as 69.60 %leaf area of infection but it was dramatically decreased after spraying with fungicides. Statistical differences in disease severity between the fungicidal treatment and the nontreated control among sunflower lines were found significantly (Table 3). When compared with the non-treated control, the combined treatment of fungicides reduced the infected leaf area by 12.20, 5.00, and 3.10 %; reduced number of lesions per stem by 62.55, 85.85, and 83.50 %; reduced number of lesions per head by 50.38, 71.67, and 37.45 % and reduced number of lesions per branch by 33.50, 28.33, and 37.45 % for Hysun 33; KU line and Synthetic #3, respectively. Differences (P = 0.05) in the mean values of infected leaf area between the nontreated control and the fungicide treatments reflected the degree of susceptibility (disease reaction) of the sunflower lines from level index 5 (susceptible) reduced to level index 4 or moderately susceptible (Table 3). However, there was no significant difference in number lesions per head among sunflower lines with fungicide treatments and nontreated control and sunflower lines x treatment and no treatment interaction except on Synthetic #3. Number lesions per head of nontreated control was highest among lines tested and was significant difference from fungicide treatments for Synthetic #3 (Table 3).

Foliar sprays contributed to a significant seed size and seed yield increases in all trials. In Table 3, the result revealed that the mean 100-seed weight of nontreated control was significantly different (P =0.01) from the fungicide treatment. The effect of Alternaria blight reduced the 100-seed weight of non-treated control with 17.22, 6.95, and 10.31 % for Hysun 33, KU line, and Synthetic #3, respectively. Contrarily, fungicide treatment increased yields by 19.75, 13.06 and 18.71 % for Hysun 33, KU line, and Synthetic #3, respectively (Table 3). Yields from fungicide treatments revealed in the sequentialy order of Hysun 33: 351.67, Synthetic #3:351.00, and KU line: 323.00, kg/rai, respectively with no statistical different among sunflower lines tested (Table 3).

Table 3 Effects of iprodione plus with mancozeb and Triton CS-7 sprays on disease severity, seed size, and seed yield of 3 sunflower lines infected naturally with *Alternaria* spp.

Sunflower lines	Treatment[1/]	Disease severity: Number of lesions per stem	per head	per branch	Percentage of infected leaf	Disease[2/] reaction	100 seeds weight (g)	Yield (kg/rai)	Yield[3/] increased (%)
Hysun 33	FS	1.00	2.00 ab	2.67	57.50	4	8.13	351.67	19.75
	NT	2.67	1.33 b	2.00	69.70	5	6.73	293.67	
KU line	FS	0.33	1.67 b	1.67	65.40	4	7.63	323.00	13.06
	NT	2.33	2.33 ab	2.33	72.10	5	7.10	285.67	
Synthetic #3	FS	0.33	1.67 b	1.67	63.80	4	7.47	351.00	18.71
	NT	2.00	2.67 a	2.67	66.90	5	6.70	295.67	
Means[4/]	FS	0.56 b	1.78ns	1.78 b	62.23 a	4	7.74 a	341.89 a	17.22
	NT	2.33 a	2.11ns	2.56 a	69.60 b	5	6.84 b	291.67 b	
F-test		19.69**	1.96ns	11.67**	7.40*	10.96**	64.52**		

ns = Non-significant; * = significant different at P = 0.05; and ** = significant different at P = 0.01 as determined by DMRT.

1/ = FS = fungicide foliar spray; NT = not treated with fungicides.

2/ = Percentage of leaf area infected with *Alternana* spp. was evaluated as same as disease reaction in Table 1 and 2.

3/ = % Yield increased = (Yield obtained from FS-yield obtained from NT) x 100/yield obtained from NT.

4/ = Average of 360 plants of three sunflower lines.

DISCUSSION

The development of Alternaria blight varied by host growth stage, time of inoculation and method of inoculation at which sunflowers were inoculated. Significant hybrid by those treatment interactions also reflected the difference between the lines with respect to the responses of plant at various factors tested. Allen *et al.* (1983) reported a significant role of plant age in the development of Alternaria blight that anthesis or seed filling stage of growth was most susceptible. The results obtained in this study supports his work that sunflower plants are more susceptible to infection by *Alternaria* spp. during the reproductive (R_1) than vegetative growth stage (V_6). The plant age response was the same for all genotypes, primarily because all hybrids tested were similar in the level of disease reaction.

The age-dependent responses observed in the experiment may have important implications for Alternaria blight management in planting that

are staggered in time, a common sunflower production practice. In years for which forcasts indicate favorable Alternaria blight development, inoculum was predicted to be abundant in mid-to late-season plantings, when moderately susceptible and susceptible hybrids are at the critical susceptible growth stages (reproductive growth stages), resulting in substantial yield reduction if plants are infected. To avoid yield losses in those years, moderately resistant and resistant hybrids should be grown at all plantings. The age dependent responses of sunflower hybrids may also affect strategies for the use of fungicides to control Alternaria blight, if young plant application of fungicides can maintain disease severity below the threshold level. Three to five applications of fungicides such as iprodione and mancozeb beginning at V_4 host growth stage, with an interval of 10-14 days, have been recommended to control Alternarla blight (Prathuangwong *et al.*, 1991).

The effect of Alternaria blight on sunflower yield increased as the age at which plants were inoculated increased. The yield of all hybrids tested were more reduced when plants were inoculated at R_1 (flowering stage) than plants inoculated at V_6 growth stage, although responses are likely to vary among environments. Sackston (1959) compared the reductions in yield components resulting from artificial defoliation at the seedling, flowering and maturing stage of sunflower plant growth and found the plant height, seed weight, protein and oil content of the seed were reduced most when plants were defoliated (severe infection) at the flowering stage. This growth stage corresponds to that at which sunflower appears to be most susceptible to Alternaria blight.

When sunflower was inoculated during 09.00-11.00 am of the day, the greatest infection, the lowest weight per 100 seeds, and the lowest yield (kg/rai) were noticed compared with plants inoculated at 19.00-21.00 pm. Investigation into the effect of day time (09.00-11.00 am) having sunlight effected sporulation and germination of *Alternaria* spp. The sunlight on leaves could stimulate the abundance of sporulation and germination of conidia on the leaf surface and thereby increase the severity of Alternaria blight during this period (Allen *et al.*, 1983; Wheeler, 1969). Another result may be because of the day time, stomata have the greatest opening enabling the pathogen to enter easily and infect the plant severely (Allington and Feaster, 1946). *Alternaria* spp. frequently invaded leaves by stomatal penetration (Droby *et al.*, 1984; Ramm, 1962). The pathogens entered the leaves abundantly caused heavy infection inducing the reduction of photosynthetic area and subsequent decreased yield. Such severe infection prevented synthesis of enough nutrient to build the seeds, resulting in seeds of small size and lower yield. The effect of time at inoculation could accentuate. The effects of other environments such as humidity, temperature, vigor of plant, virulence of the pathogen included host growth stage on disease development. If there are changes in these variables, the result obtained in this experiment might be changed accordingly.

The place inoculation technique consistently resulted in higher leaf infection than spray inoculation technique with no statistical difference but had the disadvantage of differently to apply. The spray inoculation technique was, nevertheless, faster, easier and more convenience to alpply than the place inoculation technique. Although the place inoculation technique affected higher infection than the another, it was time and labour-consumings. From this investigation, however, it still could not be concluded that the spray inoculation technique is superior to the other techniques. The most suitable technique would be shown when the spray inoculation technique was compared with the place inoculation technique for its correlation with disease severity, yield loss, convenience, easiness,

and rapidity.

The results from infection assays in these studies indicate that sunflower genotypes can be screened in field tests for resistance to infection by *A. helianthi, A. zinniae* and *A. alternata* using either spray inoculation or place inoculation at which plants are R1 growth stage and inoculated during 09.00-11.00 am.

When 3 sunflower accessions were tested under field conditions with the inoculum density of 1.5×10^3 spores/ml, no differences in disease severity could be detected among them. They were infected severely by *A. helianthi, A. zinniae* and *A. alternata* with the rating of disease reaction of susceptibility (level 5). This may indicate that the *H. annuus* genotypes may have had some degree of field plant resistance that conditions were unsatisfactory to determine the differential response observed in the field. A significant lines x treatment interaction was detected in the combined analyses for Alternaria blight, indicating expression of resistance is environmentally dependent and that caution should be exercised in interpreting disease reactions from a single environment. Increased replication and sampling of environments would be necessary to detect small differences in levels of Alternaria blight resistance among sunflower hybrids. The type of resistance observed in field tests with sunflower plant was expressed quantitatively as a reduction in percentage of leaf area affected, and there was no indications of a qualitative or lesion type resistance among the accessions tested included disease assessment keys for bract, branch, stem, flower or head infection. Sunflower may show resistant leaves and susceptible stems or bracts, etc. Until specific information is available on the inheritance of these quantitative resistances to Alternaria blight, the use of artificial inoculation methods in conjunction with a recurrent selection scheme based on some sort of accession test is probably the best strategy for developing some populations that will yield hybrid lines with high level of resistance. Because the level of resistance detected in plants were not great, or only slightly improved over those hybrids, the search for better sources of resistance in *H. annuus* should continue before starting a breeding program.

Hysun 33 appeared to be more resistant to *Alternaria* spp. compared with other two accessions. It not only had less leaf area affected than others but also gave highest yield. This result, however, was not statistical difference from those of KU line and Synthetic #3. More information is needed on the genetic and physiology of resistance in these 3 lines before the significance of lesion types or assessment key for other plant parts affected can be understood.

Foliar spray with iprodione and mancozeb plus with Triton CS-7 effectively reduced the severity of Alternaria blight and subsequently contributed to a yield increase for all lines. Iprodione and mancozeb were expected to be effective directly for Alternaria blight, whereas, Triton CS-7 was expected to be adjuvant as binder increased the effectiveness of those two fungicides. Becauses the three accessions were susceptible to Alternaria blight with no statistical differences among them and this disease had greatest influence on yield reduction. As expected, the disease that had the highest severity on a particular line was effected the most by the fungicide treatments. This points to the importance of experimenting with fungicide rates and schedules on an array of accessions over several years and locations for developing reccommendations.

LITERATURE CITED

Allington, W.B. and C.V. Feaster. 1946. The relation of stomatal behavior at the time of inoculation to the severity of infection of soybean by *Xanthomonas phaseoli* var *sojense* (Hedges)

Star. and Burk. Phytopathology 36: 385-386.

Allen, S.J., J.K. Kochman, and J.F. Brown. 1981. Losses in sunflower yield caused by *Alternaria helianthi* in southern Queensland. Aust. J. Agric. Anim. Husb. 21: 98-100.

Allen, S.J., J.F. Brown, and J.K. Kochman. 1983. Effects of leaf age, host growth stage, leaf injury, and pollen on the infection of sunflower by *Alternaria helianthi*. Phytopathology 67 : 896-898.

Carson, M.L. 1985a. Epidemiology and yield loss associated with Alternaria blight of sunflower. Phytopathology 75: 1151-1156.

Carson, M.L. 1985b. Reaction of sunflower inbred lines to two foliar diseases. Plant Dis. 69: 986-988.

Droby, S.A.D., D.D. Prusky, and R.B. Golan. 1984. Pathogenicity of *Alternaria alternata* on potato in Israel. Phytopathology 74: 537-542.

Herr, L.J., and F.E. Lipps. 1982. *Alternaria helianthi* on sunflower in Ohio. Plant Dis. 66: 509-512.

Morris, J.B., S.N. Yang, and L. Wilson. 1983. Reaction of *Helianthus* species to *Alternaria helianthi*. Plant Dis. 67: 539-540.

Prathuangwong, S., W. Charleepraom, and T. Sommartya. 1989. Sunflower diseases in some growing areas of Thailand. Kasetsart J. (Nat. Sci) 23: 287-304.

Prathuangwong, S. and C. Preecha. 1990. Development of standard method and format for measuring severity of soybean bacterial pustule. Annual report of a research seminar on high protein crops for 1987 to 1989.Held at National Agricultural Extension and Training Center, Kampaeng Saen, Nakorn Pathom, Thailand during January 8-9, 1990. 10 p.

Prathuangwong, S., S. Wongkoay, T. Sommartya, and P. Sinchaisri. 1991. Role of four *Alternaria* spp. causing leaf and stem blight of sunflower in Thailand and their chemical controls. Kasetsart J. (Nat. Sci.) 25: 112-124.

Ramm, C. 1962. Histological studies of infection by *Alternaria longipes* on tobacco. Phytopathol. Z. 45: 391-398.

Sackston, W.E. 1959. Effects of artificial defoliation on sunflowers. Can. J. Plant Sci. 39: 108-118.

Wheeler. B.E.J. 1969. Leaf spot caused by *Alternaria* spp. pp. 295-196. *In* B.E.J. Wheeler. An introduction to plant disease. John hiley & Son, Ltd., London.