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BURN-DOWN EFFECT AND CHLOROSIS OF TRANSGENIC AND CONVENTIONAL CORN VARIETIES CAUSED BY GLYPHOSATE POTASSIUM 660 G L^{-1} AT DIFFERENT TIMES OF APPLICATION

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Running title: Burn-down effect and chlorosis in corn caused by glyphosate

ABSTRACT

The purpose of this experiment was to quantify the burn-down effect and chlorosis due to different times of herbicide glyphosate potassium 660 g l^{-1} application. The experiment was performed at Agriculture Faculty Research Station of Padjadjaran University, West Java Indonesia, from December 2015 to April 2016. The experiment was arranged in randomized block design with twenty treatments and each treatment was replicated two times. The treatments were application of herbicide glyphosate potassium 660 g l^{-1} at the dose of 2 L ha^{-1} on five transgenic corn varieties (C7 RR, 979 RR, 77 RR, 85 RR, and 95 RR) and five conventional corn varieties (C7, 979, 77, 85, and 95) at 15-day after planting and 20-day after planting. The result of this experiment shows that glyphosate potassium 660 g l^{-1} was effective to control weed in both transgenic and conventional corn varieties. The variety of transgenic corn exhibited smaller percentage of chlorosis (0-20%) and no burn-down effect following applications of glyphosate potassium 660 g l^{-1}. On the other hand, chlorosis and burn-down effect were found in all conventional corn varieties. The yield of transgenic corn varieties was higher than the conventional corn varieties.

Keywords: burn-down, chlorosis, conventional corn, potassium glyphosate, transgenic corn.

INTRODUCTION

In Indonesia, corn (*Zea mays* L.) is a subordinate crop besides rice. Corn is mainly processed as food for human, also to the benefit of poultry and livestock. National corn demand in 2016 was 13.8 million tons. However, national corn production has reached an all-time high under the national needs. The national corn demand cannot be fulfilled as the low yield of corn. The presence of weed is one of significant factors. Weed can reduce crop yields through competition for nutrients, water, space, and sunlight. Potential yield loss caused by weed, specifically in corn, is estimated to range between 16 to 80% (Paller, 2002). Clay and Aquilar (1998) stated that yield loss in corn caused by weed can reach 95%, therefore the presence of weed in corn must be controlled. Culpepper and York (2000) stated that herbicide is economically important weed control method in corn; however it should be implemented carefully to avoid negative impact on corn. According to Dill (2005) transgenic corn varieties, as a result of genetic engineering, can increase yield of corn.

Corn variety, namely NK 603, is a new transgenic crop variety in Indonesia. This transgenic variety contains of a gene so called CP4 EPSPS (5-enolpyruvyl shikimate-3-phosphate synthase) which makes the corn plants tolerant to the glyphosate herbicide. The CP4 EPSPS genes are the result of the isolation of soil bacterium *Agrobacterium tumefaciens* strain CP4 (Riches and Valverde, 2002). Glyphosate is effective to control grass and broadleaf weeds. According to Klingman *et al*. (1975), foliar application of glyphosate is subjected to translocation to all parts of plant, while root absorption is negligible, in which the herbicide quickly decomposes in the soil. The poisoning symptoms, due to glyphosate, develop slowly and can be seen at 1-3 weeks after application. Leaf chlorosis and purpling veins, followed by necrosis and abnormalities symptoms such as visible white spots and stripes are all the symptoms of poisoning caused by glyphosate (Ashton and Crafts, 1981). Leaf chlorosis is caused by inhibition of the enzyme 5-enolpyruvil-shikimic-3-phosphate synthase (EPSP Synthase), which plays an important role in amino acid biosynthesis of phenylalanine, tyrosine, and tryptophan. According to Monaco *et al.* (2002) weeds will diminish slowly in 1-2 weeks after the application of glyphosate, and then weeds turn into brown color, where death is inevitable. The drift of glyphosate application on crop can be morphological defects, chlorosis, sterility, and yield loss (Heck *et al*., 2005).

The effect of glyphosate potassium herbicide at two different times of application, specifically to the resistant NK603 and conventional corn varieties, has not been known yet. Therefore, the present study aims to determine the efficacy of glyphosate potassium for weed control and evaluate the chlorosis and burn-down effect of glyphosate potassium on NK603 transgenic and conventional corn varieties.

MATERIALS AND METHODS

The experiment was conducted at Agriculture Faculty Research Station of Padjadjaran University, Jatinangor Sumedang, West Java Indonesia. The experiment was conducted from December 2015 to April 2016. The materials used in this experiment were five corn transgenic varieties (C7 RR, 979 RR, 77 RR, 85 RR, and 95 RR), five conventional corn varieties (C7, 979, 77, 85, and 95), and fertilizers (urea, phosphate, KCl), fungicides with the active ingredient Dimetomorf and Pyraclostrobin + Epoxiconazole. The insecticides used were Deltamethrin, Firpronil, and Betasiflutrin. The glyphosate potassium was applied by using semi-automatic knapsack sprayer with a pressure of 1 kg / cm3. The experimental design used was randomized block design, which consisted of 20 treatments and each treatment was replicated two times; so that there were 40 plots (Table 1). The individual plot size was 3 m x 2.8 m. Glyphosate potassium herbicide was applied at 15 and 20 days after planting with the recommended dose of 2 L ha^{-1}.

Table 1. Application of glyphosate potassium herbicide at two different times of application to transgenic and conventional corn varieties

Symbol	Corn varieties	Time of application (DAP)
A	Transgenic C7 RR	15
B	Conventional C7	15
C	Transgenic 979 RR	15
D	Conventional 979	15
E	Transgenic 77 RR	15
F	Conventional 77	15
G	Transgenic 85 RR	15
H	Conventional 85	15
I	Transgenic 95 RR	15
J	Conventional 95	15
K	Transgenic C7 RR	20
L	Conventional C7	20
M	Transgenic 979 RR	20
N	Conventional 979	20
O	Transgenic 77 RR	20
P	Conventional 77	20
Q	Transgenic 85 RR	20
R	Conventional 85	20
S	Transgenic 95 RR	20
T	Conventional 95	20

Note: DAP = Day after planting

The observations used were: 1). Weed dry weight (g) was carried out at 3 and 6 weeks after herbicide application, 2). Glyphosate injury in corn was evaluated as chlorosis symptom at 3 and 5 days after herbicide application and burn-down symptom at 7 and 14 days after herbicide application. The symptoms of burn-down was assessed by using the score system: 0 = No burn-down, 0-5%, the shape or colour of the young leaves are not normal; 1 = Mild burn-down, 6-20%, the shape or colour of young leaves are not normal; 2 = Moderate burn-down, 21-50%, the shape or colour of young leaves are not normal; 3 = Severe burn-down, 51-75%, the shape or colour of young leaves are not normal; 4 = very severe burn-down,> 75%, the shape or colour of young leaves are not normal to dry out and fall off until the plants die, 3) dry seed weight per plot (kg). All data were analysed by using Minitab statistical Program.

RESULTS AND DISCUSSION

Vegetation Analysis before applying Glyphosate Potassium Herbicide

The dominant weed species that were found on the field before spraying glyphosate potassium herbicide were three species of broadleaf: *Bidens pilosa*, L., *Cleome rutidosperma*, DC. and *Alternanthera sessili* (L) R.Br. ex DC.

Weed dry weight

Table 2 and Table 3 show the effect of timing application of glyphosate potassium herbicide 660 g l^{-1} on the transgenic and conventional corn varieties at 3 and 6 weeks after herbicide applications. A total of 20 weed species, comprising of 14 broadleaves, 5 grasses, and one species of sedge were observed in both transgenic and conventional corn fields. There was no significant difference in the dry weight of the predominant weeds (*Bidens pilosa*, *Cleome rutidosperma*, and *Alternanthera sessilis*), dry weight of other species of weeds, and dry weight of weed in total at both application times. This result indicates that the application of glyphosate potassium herbicide 660 g l^{-1}, at 15 and 20 days after planting, has similar effectiveness in controlling all weed species, disregards of their composition and dominance in the field. Other recorded weeds species were *Cyperus rotundus L*, *Commelina diffusa* Burm. f, *Mimosa invisa* Mart. ex Colla, *Panicum repens* L, *Borreria alata* (Aubl.) DC, *Mimosa pudica* L, *Phyllanthus niruri* Linn, *Phyllanthus urinaria* L, *Synedrella nodiflora* (L) Gaertn, *Emilia sonchifolia* (L.) DC.), *Digitaria ciliaris* (Retz) Koel, *Ageratum conyzoides* L, *Oxalis barrelier Li*, *Axonopus compressus* (Swartz.) Beauv., *Cynodon dactylon* (L.) Pers., *Euphorbia hirta* L, and *Imperata cylindrica* (L.) Raeusch.).

Table 2. Dry weight of dominant weed.

Symbol	Variety	Treatment DAP (days)	Weed dry weight of *Bidens pilosa* (g/0,25 m^2)		Weed dry weight of *Cleome rutidosperma* (g/0,25 m^2)		Weed dry weight of *Alternanthera sessilis* (g/0,25 m^2)	
			3 WAA	**6 WAA**	**3 WAA**	**6 WAA**	**3 WAA**	**6 WAA**
A	C7 RR	15	0,20 a	7,05 a	0,20 a	0,00 a	0,10 a	0,85 a
B	C7	15	0,30 a	0,00 a	0,30 a	0,00 a	0,40 a	4,85 a
C	979 RR	15	0,00 a	0,40 a	0,00 a	0,00 a	0,00 a	3,10 a
D	979	15	0,00 a	0,00 a	0,00 a	0,10 a	0,00 a	0,90 a
E	77 RR	15	0,35 a	2,15 a	0,35 a	0,00 a	0,30 a	0,55 a
F	77	15	0,00 a	1,60 a	0,00 a	0,10 a	0,00 a	1,70 a
G	85 RR	15	0,05 a	0,05 a	0,05 a	0,00 a	0,05 a	0,85 a
H	85	15	0,00 a	0,20 a	0,00 a	0,00 a	0,00 a	2,80 a
I	95 RR	15	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,80 a
J	99	15	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	6,40 a
K	C7 RR	20	0,50 a	0,00 a	0,50 a	0,00 a	0,60 a	1,10 a
L	C7	20	0,80 a	0,00 a	0,80 a	0,25 a	0,80 a	3,35 a
M	979 RR	20	0,05 a	0,20 a	0,05 a	0,00 a	0,05 a	0,55 a
N	979	20	0,30 a	0,40 a	0,30 a	0,00 a	0,30 a	1,25 a
O	77 RR	20	0,50 a	0,00 a	0,50 a	0,00 a	0,45 a	0,10 a
P	77	20	0,15 a	0,00 a	0,15 a	0,00 a	0,05 a	5,50 a
Q	85 RR	20	0,15 a	0,25 a	0,15 a	0,00 a	0,15 a	0,00 a

R	85	20	0,40 a	0,00 a	0,40 a	0,00 a	0,40 a	1,65 a
S	95 RR	20	0,30 a	0,10 a	0,30 a	0,00 a	0,45 a	1,20 a
T	99	20	0,85 a	3,60 a	0,85 a	0,00 a	0,95 a	0,60 a

Note: - The average value, followed by the same letter in the column, shows no significant difference (according to the Scott-Knott test at the level of 5%).
- DAP: day after planting
- WAA: week after herbicide application

Table 3. Dry weight of other Wwed species and total weed

Symbol	**Variety**	**Treatment DAP (days)**	**Total weed dry weight ($g/0,25\ m^2$)**		**Weed dry weight of others species ($g/0,25\ m^2$)**	
			3 WAA	**6 WAA**	**3 WAA**	**6 WAA**
A	C7 RR	15	0.30 a	1.05 a	0.00 b	0.20 a
B	C7	15	0.70 a	3.05 b	0.00 b	0.15 a
C	979 RR	15	0.00 a	6.00 a	0.00 b	2.50 a
D	979	15	0.00 a	1.45 a	0.00 b	0.45 a
E	77 RR	15	1.30 a	2.70 a	0.00 b	0.00 a
F	77	15	0.00 a	4.20 a	0.00 b	0.80 a
G	85 RR	15	0.20 a	1.05 a	0.00 b	0.15 a
H	85	15	1.55 a	6.75 a	0.80 a	1.47 a
I	95 RR	15	0.00 a	1.20 a	0.00 b	0.40 a
J	99	15	0.00 a	6.85 a	0.00 b	0.45 a
K	C7 RR	20	1.55 a	1.40 a	0.00 b	0.30 a
L	C7	20	1.90 a	4.25 a	0.00 b	0.65 a
M	979 RR	20	0.20 a	1.15 a	0.00 b	0.40 a
N	979	20	0.80 a	5.20 a	0.00 b	3.55 a
O	77 RR	20	1.20 a	0.40 a	0.00 b	0.30 a
P	77	20	0.40 a	7.35 a	0.00 b	1.85 a
Q	85 RR	20	0.30 a	1.75 a	0.00 b	1.50 a
R	85	20	1.35 a	2.20 a	0.00 b	0.55 a
S	95 RR	20	3.50 a	2.25 a	0.00 b	0.95 a
T	99	20	0.00 a	6.35 a	0.00 b	3.15 a

Note: - The average value, followed by the same letter in the column, shows no significant difference (according to the Scott-Knott test at the level of 5%)
- DAP: day after planting
- WAA: week after herbicide application

Chlorosis, burn-down, and yield of corn

Table 4 shows that, at 3 and 5 days after herbicide application, there was chlorosis on corn caused by herbicide phytotoxicity to the corn plants. The percentage of chlorosis in conventional corn varieties was 60-85%, whereas the percentage in transgenic corn varieties was much lower (0-20%). All conventional corn varieties exhibited higher rate of chlorosis as compared to transgenic corn varieties at 3 and 5 days after herbicide application. The reason is probably that conventional corn plants were not inserted by CP4 EPSPS genes that make corn plants resistant to spraying glyphosate potassium herbicides. According to Roberts (1977) glyphosate is easily absorbed by leaves and translocatable in plants, moving through the symplastic system. Glyphosate kills plants by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). EPSPS is a key enzyme in the shikimate biosynthetic pathway which is necessary for the production of the aromatic amino acids, auxin, phytoalexins, folic acid, lignin, plastoquinones and many other secondary products.

Table 4 and Figures 1 to 6 show that the application of glyphosate potassium at 15 and 20 days after planting displayed significantly more severe burn-down symptoms in conventional corn varieties as compared to transgenic corn varieties at 7 and 14 days after herbicide application. The burn-down score in conventional corn species is 4 (very severe burn-down), whereas in transgenic corn species, the score is 0 (no burn-down). It was also observed that all conventional corn varieties were totally killed by the application of glyphosate potassium herbicide at 15 and 20 days after planting. The symptoms of burn-down in corn plants were clearly evident on young leaves. The observable symptoms are, firstly all young leaves turned yellowish-brown like burn, followed by stunted growth, and finally dead. The application of glyphosate potassium herbicide at 15 and 20 days after planting produced only a small percentage of chlorosis (0-20%), with no visible burn-down effect on the transgenic corn varieties. The reason is probably that the transgenic corn varieties have been inserted with the CP4 EPSPS gene derived from *Agrobacterium tumefaciens* that is insensitive to glyphosate. According to Heck *et al.* (2005) transgenic corn is tolerant to glyphosate. In contrast, conventional corn varieties are sensitive to glyphosate and therefore, the use of glyphosate for weed control in non-transgenic corn field is not suggested.

Table 4. Chlorosis, burn-down, and yield of corn

Symbol	**Variety**	**Treatment DAP (days)**	**Chlorosis (%)**		**Burn-down**		**Yield of Corn**
			3 DAA	**5 DAA**	**7 DAA**	**14 DAA**	**Ton Ha^{-1}**
A	C7 RR	15	0	10	0.00 b	0.00 b	10.57 a
B	C7	15	85	85	4.00 a	4.00 a	0.00 c
C	979 RR	15	0	0	0.00 b	0.00 b	9.54 a
D	979	15	75	85	4.00 a	4.00 a	0.00 c
E	77 RR	15	0	5	0.00 b	0.00 b	9.02 a
F	77	15	85	85	4.00 a	4.00 a	0.00 c
G	85 RR	15	0	5	0.00 b	0.00 b	8.60 a
H	85	15	60	85	4.00 a	4.00 a	0.00 c
I	95 RR	15	0	5	0.00 b	0.00 b	8.14 a
J	99	15	60	85	4.00 a	4.00 a	0.00 c
K	C7 RR	20	0	0	0.00 b	0.00 b	9.56 a
L	C7	20	75	85	4.00 a	4.00 a	2.60 b
M	979 RR	20	0	0	0.00 b	0.00 b	10.68 a
N	979	20	60	85	4.00 a	4.00 a	1.10 b
O	77 RR	20	0	0	0.00 b	0.00 b	8.67 a
P	77	20	75	85	4.00 a	4.00 a	1.15 b
Q	85 RR	20	5	20	0.00 b	0.00 b	7.13 a
R	85	20	60	85	4.00 a	4.00 a	1.67 b
S	95 RR	20	0	0	0.00 b	0.00 b	9.30 a
T	99	20	60	60	4.00 a	4.00 a	2.04 b

Note: - The average value, followed by the same letter in the column, shows no significant difference (according to the Scott-Knott test at the level of 5%).
- DAP: day after planting
- WAA: week after herbicide application

The yield of transgenic corn varieties that are sprayed by herbicide glyphosate potassium herbicide 660 g l^{-1} was between 7.13 to 10.57 ton ha^{-1}. On the contrary, the yield of conventional corn varieties was significantly lower, producing yield of only between 0-2.6 ton ha^{-1} (Table 4).

Figure 1. Observation on chlorosis (1) at 3 days after application of herbicide on transgenic and (2) at 15 days after planting conventional varieties by the treatment of spraying glyphosate potassium herbicide.

Figure 2. Observation on chlorosis (1) at 5 days after application of herbicide on transgenic and (2) at 15 days after planting conventional varieties by the treatment of spraying glyphosate potassium herbicide.

Figure 3. Observation on burn-down effect (1) at 7 days after application of herbicide on transgenic and (2) at 15 days after planting conventional varieties by the treatment of spraying glyphosate potassium herbicide.

Figure 4. Observation on burn-down effect (1) at 14 days after application of herbicide on transgenic and (2) at 15 days after planting conventional varieties by the treatment of spraying glyphosate potassium herbicide.

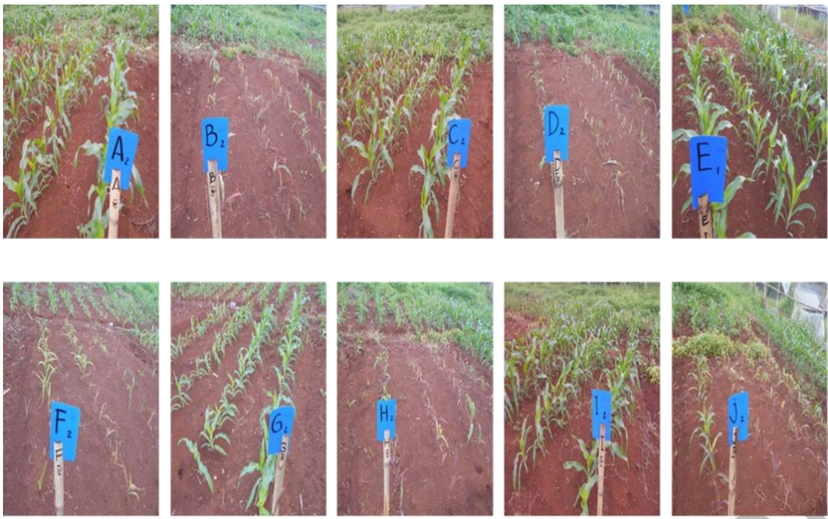

Figure 5. Observation on burn-down effect (1) at 7 days after application of herbicide on transgenic and (2) at 20 days after planting conventional varieties by the treatment of spraying glyphosate potassium herbicide.

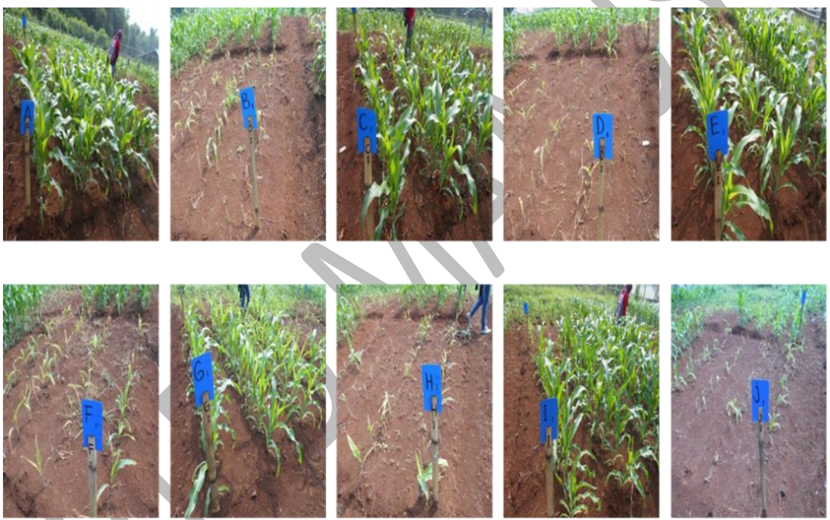

Figure 6. Observation on burn-down effect (1) at 14 days after application of herbicide on transgenic and (2) at 20 days after planting conventional varieties by the treatment of spraying glyphosate potassium herbicide.

CONCLUSIONS

All transgenic corn varieties showed small percentage of chlorosis (0-20%) and no burn-down effect following glyphosate potassium herbicide applications. Contrary to transgenic corn varieties, all conventional corn varieties displayed severe chlorosis and burn-down effect. Moreover, better weed control was evident at the glyphosate potassium application time of 20 days after planting in both transgenic and conventional corn varieties.

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