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Full Length Research Paper

Regulation of tomato (*Lycopersicon esculentum* Mill.) fruit setting and earliness by gibberellic acid and 2,4-dichlorophenoxy acetic acid application

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The experiment was conducted with the objective of determining the effects of different concentrations and combinations of the plant growth regulators (PGRs) 2,4-dichlorophenoxyacetic acid (2,4-D) and gibberellic acid (GA_3) spray on fruit setting and earliness of tomato varieties. The experiment consisted of one processing (Roma VF) and one fresh market (Fetan), tomato varieties, three levels of 2,4-D (0, 5 and 10 ppm) and four levels of GA_3 (0, 10, 15 and 20 ppm) arranged in a 2 × 3 × 4 factorial combinations, in randomized completed block design with three replications. The study indicated that application of 2, 4-D at 5 and 10 ppm hastened flowering and fruiting but reduced number of fruits per cluster, fruit set percentage and final marketable fruit number per plant. However, application of GA_3 extended flowering and maturity time and increased fruit number per cluster, fruit set percentage and marketable fruit number per plant over the control. In general, the study indicated that 2, 4-D is important in tomato production to induce fruit setting and earliness and GA_3 seems to extend fruit maturity and harvest period while the combined applications have intermediate effects. Therefore, it is important to further investigate the method of application and concentrations of these PGRs at different growing conditions and on different tomato cultivars to assess their role in tomato fruit setting and maturity time.

Key words: Gibberellic acid, 2, 4-dichlorophenoxy acetic acid, tomato, *Lycopersicon esculentum* mill, fruit setting, fruit earliness.

INTRODUCTION

In Ethiopia, tomato (Lycopersicon esculentum Mill) is an important cash crop to the farmers and a widely cultivated vegetable crop both under irrigation and rain fed throughout the year (Lemma, 2002). Tomato has a significant role in human nutrition and according to Fekadu et al. (2004), the general dietary deficiencies of vitamins in Ethiopian population could be alleviated by a liberal consumption of this vegetable because of its good source of lycopene, vitamins and antioxidants which promote a good health and removal of constipation effect. Tomato has unlimited potentialities for expansion to different agro-ecologies in Ethiopia provided that certain production constraints (low yield and quality, disease and insect pest complex, heat stress) are solved (Fekadu et al., 2003).

Fruit setting and earliness of tomato were reported to be affected by environmental, cultural or genetic factors among which light, temperature, nutrition, hormonal imbalance and water supply play a significant role (Kinet and Peet, 1997). Hence, high relative humidity of the air, low light intensity and extreme low and high temperature, and improper mineral nutrition seems to be involved in the control of those phenomena and result in low fruit set

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Abbreviations: **2,4-D**, 2,4-Dichlorophenoxyacetic acid; **GA_3,** gibberellic acid; **PGRs**, plant growth regulators.

and quality.

In the major tomato production belt of central rift valley of Ethiopia high temperature, diseases, poor irrigation practices and fertilization are some of the constraints to reduce normal vegetative and reproductive organs development for proper fruit settings and maturation. Lack of heat tolerant tomato varieties and poor fruit setting of existing varieties during the hot dry season, where the demand for tomato is very high in the market, is one of the challenges farmers are facing in tomato production during the hot dry season though there is a potential land and irrigation for increasing production. Yield loss due to the presence of insect pests like spider mite at the late harvest is also another problem in dry season tomato production. Improvement in fruit setting, fruit earliness and concentrated yield harvest might have important value to the farmers especially when commercial production is considered for processing industries in addition to reducing cost of production.

Plant growth regulators (PGRs) are used extensively in horticulture to enhance plant growth and improve yield by increasing fruit number, fruit set and size (Batlang, 2008). Earlier reports indicated increased fruit size and setting in tomato due to application of 2, 4-dichlorophenoxy acetic acid (2,4-D), 4-chlorophenoxy acetic acid (4-CPA), and β-naphthoxya cetic acid (β-NAA) (Gemici et al., 2006). Similarly, gibberellic acid (GA_3) at low concentration was reported to promote fruit setting in tomato (Sasaki et al., 2005; Khan et al., 2006). Tomato plants treated with a mixture of 4-CPA and GAs (Sasaki et al., 2005) showed increased fruit set and proportion of normal fruits compared to plants of the same crop treated with 4-CPA alone. Similarly, sprays of NAA or β-NAA at the time of flowering resulted in reduced pre-harvest fruit drop and increased the number of fruits per plant (Alam and Khan, 2002).

In general, full potential of high yielding varieties could only be realized under normal management practices, but under unfavorable conditions for tomato fruit setting, research results proved that the use of PGRs could increase both fruit setting and yield earliness. For instance a trial carried out to check the possibility of applying the ethylene releasing PGR (ETHREL) under field condition at Nura Era farm, Ethiopia, in 1984 showed that the yield collected at one picking was more than doubled regardless of the applied concentrations compared to the control (Heussler and Ayele, 1987). This clearly indicates that PGRs could improve tomato fruit earliness, fruit setting and yield under Ethiopian condition and might have commercial importance. However, these PGRs have not been exhaustively studied and there is lack of information regarding type, rate and time of application.

This study was therefore initiated to assess the effects of different concentrations and combinations of gibberellic acid and 2,4-dichlorophenoxy acetic acid sprays on fruit setting and earliness of tomato.

MATERIALS AND METHODS

The experiment was conducted in central Ethiopia, at Melkassa Agricultural Research Center, which is located at 8°24′ N latitude, 39°21′ E longitude and at an altitude of 1550 m above sea level, in the middle rift valley of Ethiopia. The center is characterized by low and erratic rainfall with mean annual rainfall of 796 mm with peaks in July and August. The dominant soil type of the center is Andosol of volcanic origin with pH that ranges from 7 to 8.2. The mean annual temperature during the study was 21.2°C with a minimum of 14°C and maximum of 28.4°C (MARC, 2008).

Two improved tomato varieties Roma VF and Fetan were obtained from Melkassa Agricultural Research Center, used as a test material. Roma VF is processing type, compact and strong stem with determinate growth habit while Fetan is fresh market type with strong stem having determinate growth habit. Commercial tissue culture grade (95% purity) and 2, 4-D (salt formulation) were obtained from Sigma, Germany.

The two tomato varieties with three levels of 2, 4-D (0, 5 and 10 ppm) and four levels of GA_3 (0, 10, 15 and 20 ppm) were arranged in a 2 × 3 × 4 factorial combinations, in RCB design with three replications. Seedlings were raised in mid September in an open nursery bed and transplanted to the experimental field after 35 days at a spacing of 30 cm between plants on ridges having 100 cm width. A net plot size of 12 m^2 (4 × 3 m) having 40 plants/plot was used. A total of 20 plants per plot, from the two middle rows, were considered for data collection. The field was irrigated using furrow irrigation when rainfall was not sufficient for plant growth. Urea as a source of nitrogen fertilizer was applied at a rate of 46 kg/ha in split form, half at transplanting and half at first flowering as a side dress, phosphorous was at a rate of 40 kg/ha all at transplanting. Weeding, cultivation and pest control were done following previous recommendations (Lemma, 2002).

The required weight of the PGRs was taken using electronic sensitive balance (model BOSCH SAE200) and a stock solution was prepared by dissolving in 1 ml of 97 percent ethanol. Latter the stock solution was diluted in distilled water (dH_2O) to prepare the working solutions, just before application. As cohesive agent, Tween-20 at a rate of 0.05% (v/v) was added in the spray solution and mixed well just before spraying. The solution was poured into hand-held atomizer sprayer and was directly sprayed on the plants at early flowering (42 days after transplanting). Spraying was performed early in the morning to avoid rapid drying-off of the spray solution, due to transpiration.

Data were collected from randomly selected plants in the two middle rows except for fruit yield where the two middle rows were considered. The collected data includes days to 50% fruiting, number of flowers and fruits per cluster, fruit set (%), marketable fruit number per plant and fruit earliness. The data was analyzed using analysis of variance (ANOVA) by SAS (2002) software and mean separation was carried out by DMRT at 5% probability level.

RESULTS

Number of fruits per cluster and fruit set percentage

Number of fruits per clusters (NFPC) were significantly different due to the interaction effects of variety with 2,4-D ($P < 0.01$) and 2,4-D with GA_3 ($P < 0.05$), indicating differential response of the tomato varieties to 2,4-D concentration (Table 1). The decreased in NFPC for Roma VF and Fetan was about 12.88 and 6.91%, respectively when the concentration of 2,4-D applied was increased from 0 to 10 ppm. This resulted in the

Table 1. Interaction effects of variety and 2, 4-D on number of fruits per flower cluster of tomato plants grown at Melkassa, Ethiopia.

Variety	2,4-D (ppm)			Mean
	0	5	10	
Roma VF	4.33[a]	3.86[b]	3.72[b]	3.97
Fetane	2.50[c]	2.44[c]	2.29[c]	2.41
Mean	3.41	3.15	3.00	
CV(%)		9.48		

Means, followed by the same letter within the table are not significantly different from each other according to DMRT at 5% probability level.

Table 2. Interaction effects of variety and 2,4-D on fruit set percentage of tomato plants grown at Melkassa, Ethiopia.

Variety	2,4-D (ppm)			Mean
	0	5	10	
Roma VF	85.89[a]	75.02[b]	76.15[b]	79.02
Fetane	52.04[c]	53.34[c]	48.74[d]	51.37
Mean	68.97	64.18	62.44	
CV(%)		9.94		

Means followed by the same letter within the table are not significantly different from each other according to DMRT at 5% probability level

development of only few numbers of flower buds that reach final fruit growth. Due to this fact, the interaction effects of variety with 2,4-D (Table 2) indicated that fruit set in Roma VF and Fetan were reduced by about 8.52 and 9.36%, respectively when the concentration of 2,4-D increased from 0 to 10 ppm. The result also indicates that Fetan was more sensitive to 2,4-D spray than Roma VF at applied concentration and resulted in less NFPC. Fruit number per flower cluster significantly reduced by 2, 4-D due to flower and/or fruit lets abscission compared with the control. In contrary with the single effects of 2, 4-D, application of GA_3 in combination with 2,4-D seems to overcome the problem of low NFPC and caused by 2,4-D alone (Table 3). The interaction effects of 2, 4-D and GA_3 had significant effect on fruit set (%). The result indicates that fruit set (%) was increased from 54.75 to 75.43% (about 21%) when GA_3 concentration increased from 0 to 15 ppm while the concentration of 2,4-D was kept at 0 ppm and decreased in the presence of 2,4-D. Yet, there is a slight increase in fruit set (%) when 2, 4-D and GA_3 were combined (Figure 1).

Marketable fruit number per plant

Interactions effects of variety and PGRs significantly ($P < 0.001$) affected marketable fruit number (MFN) per plant. Increased concentration of 2, 4-D from 0 to 5 ppm resulted in decreased MFN per plant from 42.79 to 33.42 by about 22% and further increased the concentration of 2, 4-D to 10 ppm decreased MFN per plant to 26.92 by about 37% (data not shown). On the contrary, increase in concentration of GA_3 significantly increased MFN per plant even though it starts to decline at higher concentration. The interaction effects of variety and the two PGRs (Table 4) indicated that GA_3 application resulted in an increased MFN per plant in the absence of 2, 4-D but when combined with 2,4-D, MFN per plant significantly reduced up to half for both varieties depending on increased concentration of 2,4-D. However, the rate of decrease was not as high as that of 2,4-D when applied alone.

Fruit earliness

Fruit earliness was calculated from multiple harvests divided by total marketable harvest. The result indicated that 2, 4-D application at 10 ppm resulted in early fruit maturity and maximum pick harvest in the second and third harvests while application of GA_3 alone and the control treatment extended maturity period and frequency of harvesting (Figure 1). Stimulation of extended growth and subsequent reproductive organ formation in tomato plants treated with GA_3 were observed. This treatment extended fruit maturity period and subsequent harvesting compared to plants treated with 2,4-D. Hence, improvement in fruit earliness by application of 2,

Table 3. Interaction effects of 2,4-D and GA_3 on number of fruit per flower cluster and fruit set (%) of tomato plants grown at Melkassa, Ethiopia.

2,4-D (ppm) (A)	GA_3 (ppm) (B)	Number of fruits^{-1} cluster	Fruit set (%)
0	0	2.93^{c}	54.75^{f}
	5	$3.53a^{b}$	71.10^{b}
	10	3.69^{a}	75.43^{a}
	20	$3.51a^{b}$	70.50^{b}
5	0	3.14^{a-c}	60.86^{e}
	5	3.13^{a-c}	66.31^{c}
	10	3.18^{a-c}	66.06^{cd}
	20	3.14^{a-c}	63.49^{de}
10	0	2.84^{c}	60.79^{e}
	5	3.11^{a-c}	63.68^{de}
	10	3.00^{bc}	63.27^{de}
	20	3.06^{a-c}	62.04^{e}
Mean		3.19	64.86
CV (%)		9.48	9.94
F-test	A	***	*
	B	**	***
	A × B	**	*

*, **, *** significant at 5, 1 and 0.1% level of probability, respectively; ns-non significant at 5% probability level; means followed by the same letter within the table are not significantly different from each other according to DMRT at 5% probability level.

4-D might have commercial importance when concentrated yield is considered particularly for processing industries, in addition to reduced cost of harvesting and escape disease that could emerg due to extended harvesting period.

DISCUSSION

Number of fruits per cluster and fruit set percentage

It was noted that exogenous auxin could sometimes enhance senescence and abscission in various plant organs due to its effect on increased ethylene production (Abeles and Rubinstein, 1964; Morgan and Hall, 1964; Brown, 1997). Enhancement of ethylene production by 2, 4-D in Red Cestrum cut flower stalks was also reported (Abebie et al., 2007). Serrani et al. (2007) indicated that high doses of 2,4-D resulted in some malformations in tomato which were apparently similar to those malformations described for over expression of IAA-biosynthesis genes (Pandolfini et al., 2002), while small epidermal protuberances that were never found in pollinated and GA_3 induced fruits were produced.

Effect of PGRs on NFPC is a function of time of application, crop growth stage and type of PGRs was used (Naeem et al., 2001). Furthermore, the author highlighted reduced fruit drop due to GA_3 spray on tomato plant contributed to better number of fruits per plant. Hence, application of GA_3 seems to stimulate growth of tomato plant and resulted in an increased NFPC even though there is extended fruit maturity. For instance, Gemici et al. (2000) indicated that 10 ppm GA_3 treated tomato plants showed a 17% increase in stem length when compared to the control and it is quite effective in increasing fruit size, especially in grapes (Graham and Ballesteros, 1980). Brown et al. (2005) also indicated that single applications of very small amount of GA_3 to seeds or seedling roots proved to be capable of changing growth rates of leaves and trusses.

From morphological observation, we were able to examine stimulation of growth and subsequent reproductive organ formation in tomato plants treated with GA_3 compared to plants treated with 2,4-D. In line with this finding, previous studies indicated an increase in tomato plant height following application of GA3 (Muzzucato et al., 1998). Even after the end of GA_3 treatment there was a positive effect on petal elongation and inflorescence stalk length both in wild type and pat mutants of tomato plants (Muzzucato et al., 1998). The extent of hormone and temperature induced floral modifications also depends on the genetic back-ground (Sawhney, 1983) which corroborates the difference observed between Roma VF and Fetan. It has been suggested that the GAs produced by the pollen may play a role in increasing auxin production in the ovary, which may serve as a signal for fruit set and further cell division (Gillaspy et al., 1993). High GA levels have been detected in young, immature tomato fruits (Koornneef et al., 1990) which may play a role in anthesis and stimulate fruit and seed development (Rebers et. al., 1999). It was also indicated that there was a probability that mixtures of auxin with other growth regulators such as GAs promote tomato fruit set and development (Schwabe, 1986). Therefore, this present finding of increased fruit set due to GA_3 could be by stimulation of plant growth and reproductive organs formation. The synthetic auxin 2,4-D mimics the function of natural auxins which control “a multitude of plant growth and development processes” (Hess, 1993). Concentrations of auxins normally fluctuate in order to properly direct growth. In cells exposed to 2,4-D, however, levels of this auxin remain high because 2,4-D is more stable and persistent than auxins (Hess, 1993; Zimdahl, 1993). It seems, therefore, that low concentrations of 2, 4-D below 5 ppm could improve plant growth and fruit set, while higher concentrations have herbicidal effect.

Marketable fruit number per plant

Application of PGRs (especially under low pollen viability

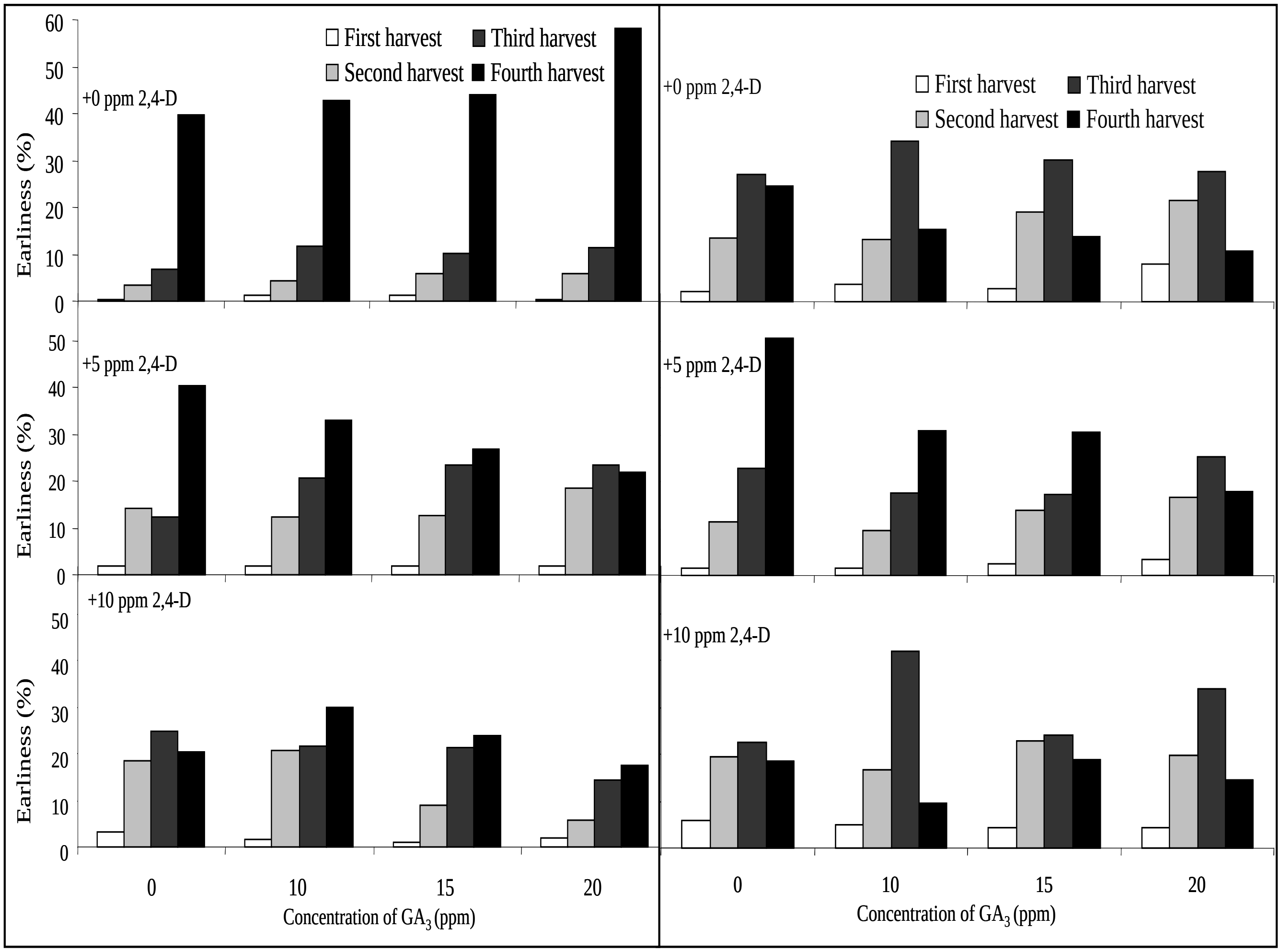

Figure 1. Influence of 2,4-D and GA_3 spray on marketable fruit earliness (%) of tomato variety Roma VF (left) and Fetan (right) grown at Melkassa, Ethiopia.

Table 4. Interaction effects of variety, 2,4-D and GA_3 on marketable fruit number per plant of tomato plants grown at Melkassa, Ethiopia.

2,4-D (ppm)	Roma VF				Mean	Fetane				Mean
	GA_3 (ppm)					GA_3 (ppm)				
	0	10	15	20		0	10	15	20	
0	41^{ef}	62^{a}	55^{b}	55^{b}	53	28^{c}	35^{g}	33^{gh}	33^{g}	32
5	47^{cd}	49^{c}	48^{c}	40^{f}	46	21^{j}	21^{j}	20^{j}	22^{j}	21
10	39^{f}	34^{g}	44^{de}	30^{hi}	37	19^{j}	14^{k}	15^{k}	20^{j}	17
Mean CV(%)	42	48	49	42	10.97	23	24	23	25	

Means, followed by the same letter within the table are not significantly different from each other according to DMRT at 5% probability level.

and fertilization) plays a role in inducing parthenocarpy and/or supporting the development of naturally induced parthenocarpy to develop and attain maximum MFN and size. Corella et al. (1986) indicated that failure of fruit swelling to reach marketable fruit size can be reduced by applying synthetic auxins and GA, which improve fruit growth thereby increasing the percentage of MFN per plant. An increased MFN per plant due to GA_3 application observed in this study also hold the finding of Khan et al. (2006) who stated that GA_3 at 10^{-8}, 10^{-6} and 10^{-4} M proved to induce higher number of fruit per plant than the untreated control. On the other hand, application of Gas can cause fruit set and growth of some fruits, in cases where auxin may have no effect (Taize and Zeiger, 2002). It was also reported that GA_3 can prevent flower abortion caused by low light intensities and enhance latter development of flower buds (Nester and Zeevaart, 1988). The significant effect of GA_3 in tomato plant further explained via its role in synthesis of protein including various enzymes (Ballesteros, 1980); increased rate of shoot elongation and photosynthetic capacity leading to total leaf area and leaf dry weight (Ballantyine, 1995; Mostafa and Saleh 2006).

The effects of 2, 4-D at high concentration resulted in fewer fruits per plant than GA_3 and the control treatments. Exposure of plants to high dose of 2,4-D spray resulted in low MFN per plant due to high rate of flower bud abscission but increased in fruit length and width due to reduced competition for assimilate among the reaming fruits. Our result also supported the findings of Gemici et al. (2006) that suggested high concen-trations of 2,4-D at 10 ppm produced fewer fruits than with 4-CPA.

Fruit earliness

Stimulation of extended growth and subsequent repro-ductive organ formation in tomato plant treated with GA_3 was observed which extended fruit maturity period and subsequent harvesting compared to plants treated with 2,4-D. Hence, improvement in fruit earliness by application of 2, 4-D might have commercial importance when concentrated yield was considered for processing industries in addition to reduced cost of harvesting and escape post disease emergency due to extended harvesting period.

Conclusion

Application of 2, 4-D at 5 and 10 ppm resulted in reduced plant growth and hastened early flowering and fruiting with concentrate pick harvest. On the contrary, GA_3 applications at all concentrations seem to promote vegetative growth and extended maturity time and harvest. Improvement in number of fruits per cluster, fruit set, and marketable fruit number per plant due to GA_3 was observed. This suggested that herbicidal effect of 2,4-D at higher concentrations hastened maturity of the plants so that they complete their life cycle in a short period of time while GA_3 spray seems to extend growing and maturity time. In general, these findings suggested that both PGRs have opposite effect in fruit setting, fruit number per plant and fruit earliness. Therefore, based on market preference and cost of production, it is possible to induce fruit earliness without affecting the final yield and quality by application of 2, 4-D and vice versa. However, these synthetic substances should be used only at tested and recommended concentrations in order to preserve quality crop product that are safe and healthy for the consumer instead of focusing on short term gains of high yield. Method of application should also be considered as spray flowers only seems to be more important than applying on the whole plant in the case of 2,4-D to avoid the herbicidal effect on the leaf part.

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