

## Mode of Action of Several Surfactants on Paraquat Efficacy

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**Abstract** : The effects of 24 ionic and nonionic surfactants on paraquat (1, 1'-dimethyl-4, 4'-bipyridinium) efficacy were investigated with several annual plant species under greenhouse conditions. The paraquat efficacy was decreased or even lost when treated with the anionic surfactants tested. However, the efficacy of paraquat was significantly increased by 7 nonionic surfactants such as sorbitan palmitate, sorbitan stearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan monostearate, polyoxyethylene stearyl ether, polyoxyethylene laurylamine ether, and polyoxyethylene stearylamine ether. Among these tested surfactants, 0.08% of polyoxyethylene laurylamine ether most significantly increased the paraquat activity, and the GR<sub>50</sub> value of paraquat with polyoxyethylene laurylamine ether was 1.6 times lower than the GR<sub>50</sub> value without polyoxyethylene laurylamine ether. In *in vitro* experiments, cellular leakage and chlorophyll contents between the application with and without polyoxyethylene laurylamine ether did not show significant changes. The absorption rate of <sup>14</sup>C paraquat in the treatment with polyoxyethylene laurylamine ether showed an absorption rate of 1.6 times higher than without surfactant. These results suggest that using compatible surfactants would increase the paraquat efficacy, and this increasing are due to improved absorption rate with the surfactant. (Received August 20, 2002; accepted September 30, 2002)

Key words : absorption, <sup>14</sup>C-paraquat, polyoxyethylene laurylamine ether, surfactant.

### Introduction

Surfactants are commonly used in post-emergence herbicide treatments to increase the efficacy of active ingredients. They can modify the physico-chemical characteristics of a spray solution, thus increasing wetting and coverage on a plant surface, and enhance penetration of active ingredient into the plant tissues. The addition of nonionic surfactant to pyridate increased control of prickly sida

(*Sida spinosa*) and common cocklebur (*Xanthium strumarium* L.) over pyridate alone (Edenfield *et al.*, 2001). Adjuvants combined with quinclorac provided significantly greater control than quinclorac alone (Zawierucha and Penner, 2001), and paraquat at 0.09 to 0.72 Ib/A, without a surfactant, failed to control downy brome (*Bromus tectorum* L.), but with surfactant added, paraquat at 0.72 Ib/A gave control. Consideration of physical and chemical data for the surfactants suggests that efficiency of surfactants with paraquat may be associated with water solubility and hydrophilicity (Evans and Eckert, 1965). Surfactants enhanced performance of

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diuron, linuron, and bromacil (Hill, 1965). Increasing the surfactant concentration caused increases in injury with lower rates of glyphosate (Riechers, 1995) and diuron (McWhorter, 1963).

However, surfactants do not always enhance the action of herbicide. They may have no effect or even be detrimental, depending on many factors such as organic solvent, particle size, molecular structure, charge, HLB. The nature of surfactant action is complex and not totally understood. The specific interactions between herbicides and surfactants may occur at interfaces, altering both physicochemical properties and herbicidal performance (Foy, 1989). Surfactant effectiveness is dependent to some extent on HLB (hydrophilic lipophilic balance), chemical type, and molecular size of surfactant (Wyrill III, 1977), and surfactant action was related to contact angle or surface tension (Foy, 1965 ; Furmidge, 1959). However, the herbicide effect and the mechanism of surfactants were not clearly explained because these involve in the study of surfactant/active ingredient/cuticle interactions (IUPAC, 1995). Also, surfactants may be classified according to anionic, cationic, nonionic and amphoteric with ionization characteristics in water. Surfactants must be carefully selected to match application needs. Most herbicides require surfactants to maximize their efficacy or utility.

Therefore, this study was conducted to identify surfactants increasing paraquat (1,1'-dimethyl-4,4'-bipyridinium) efficacy and to determine the mechanism for the increase using annual plant species including cucumber under greenhouse and *in vitro* conditions.

## Materials and Methods

### Plant material

For whole plant experiments 13 plant species including 6 grass and 7 broad leaf species, were sown in a plastic pot containing commercial seed bed soil (Boo-Nong Soil, Seoul, Korea) and grown

in a greenhouse at 30/20±3°C, day/night temperature with about 14h photoperiod for 14 days. Grass species include corn (*Zea mays* L.), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum* L.), fall panicum (*Panicum dichotomiflorum*), large crabgrass (*Digitaria sanguinalis*), quackgrass (*Agropyron smithii*) and broad leaf species cucumber (*Cucumis sativus* L.), soybean (*Glycine max.*), cotton (*Gossypium indicum*), black nightshade (*Solanum nigrum* L.), indian jointvetch (*Aeschynomene indica* L.), velvetleaf (*Abutilon avicennae*), and cocklebur (*Xanthium strumarium* L.). For further study after the above experiments, cucumber was selected and grown under the same conditions as the above.

### Chemicals

Paraquat (1,1'-dimethyl-4,4'-bipyridinium) was obtained from Sigma Chemical Co. (St. Louis, USA). Radiolabeled paraquat (sp act 1.76 GBq/mmol) was obtained from Zeneca Agricultural Products (London, UK). Surfactants were obtained from manufactures as listed in Table 1.

### Application

In whole plant experiments, 125 µg/ml of paraquat in water solution with or without surfactants was sprayed onto plants at 14 days after seeding as previously described (Yu, 1989). Spraying was conducted with a laboratory spray gun delivering spray volume of 5,000 liters/ha. After application, the plants were placed in a vented cabinet to dry before being returned to the greenhouse. The plants were allowed to grow for 3 days and then herbicidal activity and phytotoxicity were measured visual injury.

### Cellular leakage

Cellular leakage was determined periodically by detection of electrolyte leakage into bathing medium using a conductivity meter (Denki Kagaku Keiki Co., Ltd., Musashino, Japan) as previously described (Kenyon, 1985). Cotyledons of cucumber

Table 1. Surfactants used in this study

No.	Abbreviation	R <sup>a)</sup>	Chemical name	Remarks
1	Span 20	C12	Sorbitan monolaurate	Nonionic
2	Span 40	C16	Sorbitan palmitate	"
3	Span 60	C18	Sorbitan stearate	"
4	Span 80	C18	Sorbitan oleate	"
5 <sup>b)</sup>	Tween 20	C12	Polyoxyethylene sorbitan monolaurate	"
6 <sup>b)</sup>	Tween 40	C16	Polyoxyethylene sorbitan monopalmitate	"
7 <sup>b)</sup>	Tween 60	C18	Polyoxyethylene sorbitan monostearate	"
8 <sup>b)</sup>	Tween 80	C18	Polyoxyethylene sorbitan monooleate	"
9	LE-2.8	C12	Polyoxyethylene lauryl ether	"
10	SE-10	C18	Polyoxyethylene stearyl ether	"
11	OE-8	C18	Polyoxyethylene oleyl ether	"
12	OA-14	C18	Polyoxyethylene oleyl ester	"
13	NP-10	C9	Polyoxyethylene nonylphenyl ether	"
14	SP	C8	Polyoxyethylene styrylphenyl ether	"
15	POP		Polyoxyethylene polyoxypropylene aryl ether	"
16 <sup>b)</sup>	LN-7	C12	Polyoxyethylene laurylamine ether	"
17 <sup>b)</sup>	SN-15	C18	Polyoxyethylene stearylamine ether	"
18 <sup>b)</sup>	SDSS		Sodium dioctylsulfosuccinate	Anionic
19 <sup>b)</sup>	SBNS		Sodium bisnaphthalene sulfonate	"
20 <sup>b)</sup>	SLAS	C12	Sodium lauryl sulfate	"
21 <sup>b)</sup>	PAS	C12	Polyoxyethylene lauryl sulfate	"
22 <sup>b)</sup>	ANSFC		Alkyl naphthalene sulfonate formalin condensate	"
23	CaDDBS	C12	Calcium dodecylbenzene sulfonate	"
24 <sup>b)</sup>	NPP	C9	Polyoxyethylene nonylphenyl phosphate	"

<sup>a)</sup>Number of alkyl carbon atoms.

<sup>b)</sup>Water soluble surfactant.

plants were punched into 7 mm diameter discs using a cork borer and then placed in a 6 cm diameter polystyrene Petri dish containing 7 ml of 1% sucrose, 1 mM 2-(N-morpholino) ethanesulfonic acid (pH 6.5) with or without paraquat (1  $\mu$ M) and polyoxyethylene laurylamine ether (0.08%). The leaf discs were incubated in a growth chamber at 25°C in darkness for 12 hrs and then exposed to continuous light of 120  $\mu$ mol/m<sup>2</sup>/s photosynthetically active radiation (PAR) for various time periods. All treatments for each measurement were triplicated. Because of differences in background conductivity of different treatment solutions, determinations were expressed as changes in conductivity upon exposure to light.

### Uptake

In [<sup>14</sup>C] paraquat experiment, fully expanded second leaf of cucumber plants was punched into 1 cm diameter leaf discs using a cork borer and transferred into a 6 cm diameter polystyrene Petri dish containing 1 ml of distilled water with filter paper (Whatman No.2). Abaxial surface of the leaf disc was treated with 10  $\mu$ l of [<sup>14</sup>C] paraquat (0.094  $\mu$ Ci) with and without polyoxyethylene laurylamine ether (0.08%). The treated samples were kept in the growth chamber at 150  $\mu$ mol/m<sup>2</sup>/s PAR at 25°C. At 2, 4, 6 hours after treatment, the samples were taken and washed with distilled water and chloroform. The amount of [<sup>14</sup>C] paraquat penetrated into plant was estimated by

subtracting the amount of [ $^{14}\text{C}$ ] paraquat washed out from the amount of applied. Radioactivity in the washes was counted using a liquid scintillation counter (Beckman, LS 6500).

## Results and Discussions

### Effect of several surfactants on paraquat efficacy

Dose-response curves of paraquat for the 6 grass species and 7 broadleaf species revealed that most plant species were controlled more than 90% with paraquat alone, and dose-response pattern appeared to be application rate dependent (data not shown). However, paraquat efficacy was relatively higher to the broadleaf species than the grass species. Among the test plants, we selected the cucumber plants for further experiments because of its high responsibility to paraquat more in comparison with other test plants.

To test the effect of surfactants on paraquat efficacy, paraquat efficacy in controlling cucumber plants was compared when with or without surfactants. The efficacy of paraquat showed various patterns according patterns (Fig. 1).

Seven surfactants among nonionic surfactants, polyoxyethylene laurylamine ether, sorbitan palmi-

tate, sorbitan stearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan monostearate, polyoxyethylene stearyl ether, polyoxyethylene stearylamine ether, enhanced activity of paraquat. Paraquat efficacy was increased from 60% without surfactant to about 90% with these surfactants in visual rating. For example, application of paraquat with 0.25% of polyoxyethylene sorbitan monopalmitate increased herbicidal activity from 60% to 91.1% as visual injury. However, application with 0.5% and 0.125% of the polyoxyethylene sorbitan monopalmitate showed about 71.7% and 76.7% of visual injury, respectively. Similarly, although 1% of polyoxyethylene stearyl ether increased paraquat herbicidal activity to 90.5% polyoxyethylene stearyl ether lower than 0.5% showed no increase in paraquat activity. Polypxyethylene sorbitan monolaurate and polyoxyethylene sorbitan monooleate did not increase paraquat efficacy. Moreover, the addition of sorbitan monooleate even decreased the paraquat.

Paraquat contact with the leaf was very poor when treated with most anionic surfactants except calcium dodecylbenzen sulfonate and polyoxyethylene nonylphenyl phosphate. Thus, paraquat efficacy was decreased by the addition of anionic surfactants tested (Fig. 2)., Particularly, sodium bisnaphthalene sulfonate and alkyl naphthalene sulfonate (formalin condensate), resulted in the total loss of paraquat efficacy.

These results suggested that the application of paraquat with anionic surfactants should be avoided (Guh *et al.*, 1998).

The reason for the loss of activity may be because the divalent paraquat cation binds to the anionic charge of the surfactants. These results suggested that the effect of surfactant on the herbicide activity might be quite variable and difficult to predict (Wyrill III, 1977).

Therefore, surfactants and their concentrations for enhancement of paraquat efficacy need to be well selected.

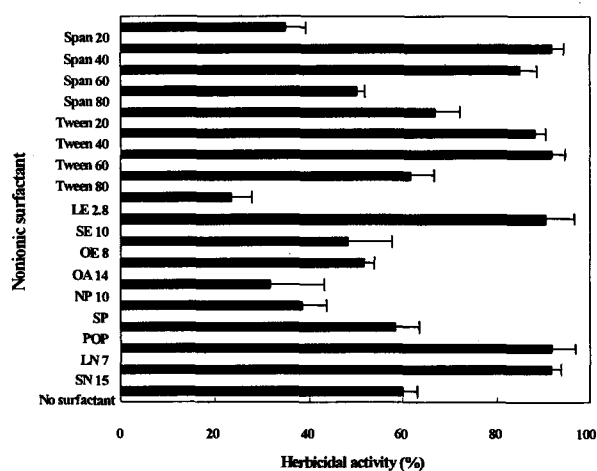


Fig. 1. Effect of nonionic surfactants on the herbicidal activity of paraquat to the cucumber plants treated as a mixture of foliar application.

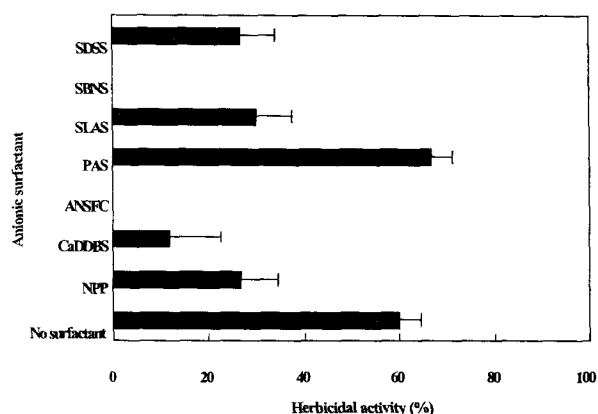


Fig. 2. Effect of anionic surfactants on the herbicidal activity of paraquat to the cucumber plants treated as a mixture of foliar application.

#### Effect of polyoxyethylene laurylamine ether (LN-7) for paraquat efficacy

The concentration of LN-7 itself inducing phytotoxicity on the test plants was very restricted. At concentrations below 0.08%, LN-7 was not phytotoxic but showed slight phytotoxic symptoms on

several test plants at 0.16%. At 0.3% most test plants except corn and quackgrass was almost damaged, and all test plants were severely damaged at over 0.6% (Table 2). Regarding contact property of paraquat on the leaf surface, it was poor at the concentrations less than 0.02% of LN-7. Therefore, these results suggest that the LN-7 concentration for enhancement of paraquat efficacy may be 0.04~0.16%. Main symptoms of phytotoxicity were chlorosis, desiccation, necrosis, and growth inhibition.

Paraquat efficacy was significantly increased when treated with addition of LN-7 on cucumber plants (Table 3). Application of 16 g/ml paraquat did not showed any herbicidal activity when treated without surfactants. However, paraquat efficacy was increased to 40% when applied with 0.02% LN-7. The herbicidal activity was increased from 20% to 100% in concentration-dependent

Table 2. Phytotoxicity of polyoxyethylene laurylamine ether (LN-7) on the test plants with foliar application

Test plants	LN-7 concentrations (%)					
	0.04	0.08	0.16	0.31	0.63	1.25
	---- % inhibition <sup>a)</sup> ----					
Cucumber	0	0	5	40	72	98
Soybean	0	2	20	50	68	92
Cotton	0	0	15	40	62	77
Corn <sup>b)</sup>	0	0	0	0	47	70
Sorghum <sup>b)</sup>	0	0	10	50	60	93
Wheat <sup>b)</sup>	0	0	0	30	55	90
Fall panicum <sup>b)</sup>	0	0	5	8	30	40
Large crabgrass <sup>b)</sup>	0	0	8	33	68	92
Quackgrass <sup>b)</sup>	0	0	0	0	45	50
Black nightshade	0	5	27	40	88	98
Indian jointvetch	0	2	20	38	78	90
Velvetleaf	0	0	0	10	70	98
Cocklebur	0	0	0	10	60	95

<sup>a)</sup>Data recorded 3 days after treatment and values represented as % inhibition with visual injury of the check based on a scale of 0 to 100, 0 = no inhibition and 100 = complete inhibition.

<sup>b)</sup>Grasses

**Table 3. Herbicidal activity of paraquat foliar application with and without polyoxyethylene laurylamine ether (LN-7) on cucumber plants**

Paraquat ( $\mu\text{g/ml}$ )	LN-7 concentrations (%)					
	0	0.02	0.04	0.08	0.16	0.32
	% inhibition <sup>a)</sup>					
0	0	0	0	0	10	20
16	0	40	40	40	40	40
32	40	50	50	50	60	70
63	50	70	70	70	70	70
125	65	80	85	90	93	100

<sup>a)</sup>Data recorded 3 days after treatment and values represented as % inhibition with visual injury of the check based on a scale of 0 to 100, 0 = no inhibition and 100 = complete inhibition.

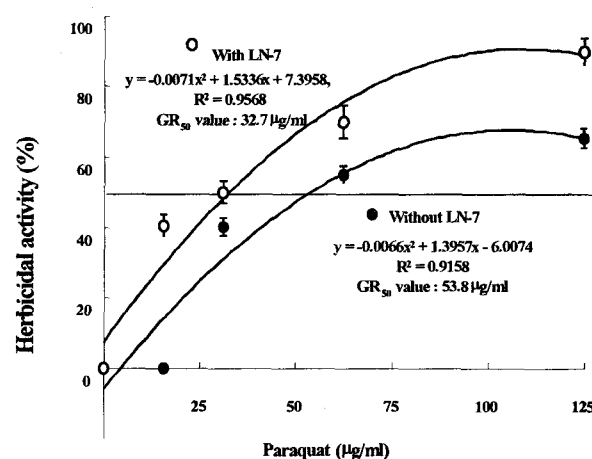
manner with LN-7 and paraquat concentrations increasing. However, in some cases, paraquat efficacy was not continuously increased with increasing of LN-7. Addition of 0.02% LN-7 to the 63  $\mu\text{g/ml}$  of paraquat showed 20% increase of paraquat activity, but the efficacy was not increased any more with increasing of LN-7 concentration until 0.32%. These results suggested that the best combination of application rate and surfactant might be restricted to the target weed, and the selection of the best combination of herbicide-surfactant should be useful for effective weed management. The  $\text{GR}_{50}$  values of paraquat application with and without LN-7 were 32.7  $\mu\text{g/ml}$  and 53.8  $\mu\text{g/ml}$ , respectively. In terms of  $\text{GR}_{50}$  values, application of paraquat with LN-7 increased its efficacy by 65% as compared with that without LN-7 (Fig. 3).

#### Effect of cellular leakage

Significant differentiation of cellular leakage was not occurred from cucumber cotyledon discs treated with 1  $\mu\text{M}$  concentration of paraquat within 6 hrs under light conditions after 12 hrs of dark incubation. Conductivities were 63.3  $\mu\text{mho/cm}$  and 57.3  $\mu\text{mho/cm}$  at 6 hours after application of paraquat with LN-7 and without, respectively (data not shown).

Generally, paraquat competes for electron with the primary electron acceptor of photosystem I and

is reduced by such electrons forming paraquat radical. The paraquat radical transfers these electrons to molecular oxygen producing several oxygen radical species, which are very reactive and toxic (Devine, 1993; Dey, 1997; Kirwood, 1991).



**Fig. 3. Dose-response of paraquat with and without polyoxyethylene laurylamine ether(0.08%, LN-7) on cucumber plants as a mixture of foliar application.**

If the enhancement of paraquat efficacy may be occurred from target site by the addition of LN-7, the significant differentiation of the membrane disruption between applications with and without LN-7 might be shown.

These results suggest that the enhancement of paraquat efficacy was not due to the interaction with LN-7 at the target site.

**Table 4. Percent distribution of applied  $^{14}\text{C}$  paraquat in the cucumber leaf disc with and without polyoxyethylene laurylamine ether (LN-7)**

Treatment	Exposure (hrs)	$^{14}\text{C}$ Paraquat (% of applied)		
		Aqueous wash	Chloroform wash	Amount percentrating cuticle
Paraquat alone	2	75.0	4.3	20.7
	4	71.9	3.2	24.9
	6	64.9	3.8	31.3
Paraquat with LN-7	2	65.8	3.9	30.3
	4	56.7	3.4	43.3
	6	47.1	3.8	49.1

**Effect of uptake**

Table 4. showed the percent distribution of applied  $^{14}\text{C}$  paraquat in the cucumber leaf discs with and without LN-7. When  $^{14}\text{C}$  paraquat was applied to the leaf disc, the  $^{14}\text{C}$  paraquat was rapidly absorbed until 6 hours after treatment. Amount of paraquat penetrated in cuticle was increased about 1.6-fold more in treatment with LN-7 than without LN-7. At 6 hours after treatment, distribution of  $^{14}\text{C}$  paraquat without LN-7 in leaf surfaces, wax layer and cuticle was 65%, 4 % and 31%, respectively. In the case of treatment with LN-7, however, distribution of  $^{14}\text{C}$  paraquat was 47%, 4% and 49%, respectively. This results indicated that enhancement of paraquat efficacy was by LN-7 due to increased penetration of paraquat into the epicuticular wax and cuticle.

For effective weed management with herbicide, in generally, herbicide active ingredient would be fully absorbed within cell membrane through the epicuticular wax and cuticle. The enhancement of paraquat efficacy with LN-7 was probably due to different penetrating amount of paraquat active ingredient through the cell membrane, since no significant differences cellular leakage and chlorophyll loss found within with/without LN-7. It has been reported that surfactants were alter epicuticular wax morphology and damage leaf tissue (Knoche, 1992), and the enhancement of herbicide toxicity by many surfactants has been

often been attributed to reduced surface tension and associated increases in leaf wettability and cuticle penetration (Wyrill III, 1977).

In conclusion, our results suggest that using compatible surfactants would increase the paraquat efficacy, and the reason for this enhancement might be due to improve absorption amount of active ingredient with the surfactant (LN-7). However, Dexter *et al.* (1966) reported that atrazine plus surfactant toxicity increased with higher relative humidities, and several surfactants alter the structure and permeability of bacterial membranes following removal of the bacterial wall with penicillin (Kiho, 1964). Also, Wyrill III *et al.* (1977) pointed out that surfactant effectiveness is dependent to some extent on surfactant HLB, chemical type, and molecular size, and Dunne *et al.* (1994) reported that the experimental adjuvant provided equal or greater uptake and translocation. Therefore, although we concluded above result, these speculations are supposed to be evaluated in our further research.

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**Paraquat 활성에 미치는 계면활성제의 작용기구**

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**요약** : 24개의 계면활성제를 대상으로 하여 paraquat의 효력에 미치는 영향을 온실조건에서 1년 생 잡초를 대상으로 조사하였다. Paraquat에 음이온 계면활성제를 혼합 처리하면 paraquat의 효력이 감소하거나 완전히 상실되었지만, sorbitan palmitate, sorbitan stearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan monostearate, polyoxyethylene stearyl ether, polyoxyethylene laurylamine ether, 및 polyoxyethylene stearylamine ether 등 비이온 계면활성제를 혼합 처리한 경우에는 paraquat의 활성이 크게 증가되었다. 이들 중 polyoxyethylene laurylamine ether를 0.08%로 혼합 처리하였을 때 가장 높은 활성을 나타내었고 혼합처리하지 않은 것에 비하여 1.6배 낮은  $GR_{50}$  값을 나타내었다. 오이 잎을 이용한 *in vitro* 실험에서 paraquat에 polyoxyethylene laurylamine ether를 혼합 처리하여도 세포질 유출량은 차이를 보이지 않았지만,  $^{14}C$  - paraquat 의 cuticle층 내로 침투되는 속도는 1.6배 높게 나타나 polyoxyethylene laurylamine ether를 혼합 처리하였을 때 paraquat의 활성이 증가하는 주원인으로 생각되었다.

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