CHAPTER 5: RESULTS AND DISCUSSION

CONTACT PHYTOTOXICITY STUDY

The entire data set of results for contact phytotoxicity can be found in Appendix A, Tables 25-30.

It is known that there is considerable inter-species variation in susceptibility to triclopyr by sweetgum, red oak and red maple, part of which may be due to contact phytotoxicity by the specific formulation and their additives. Contact phytotoxicity is the damage caused to leaf tissue (including cell death) after spray droplet contact and is not directly attributable to the active ingredient alone. It can develop quite rapidly, so a series of observations are necessary to characterize its rate and severity. Since contact phytotoxicity may be caused by high localised concentrations of formulant chemicals on a leaf surface, the addition of the organosilicone surfactants Silwet L-77 and Silwet 408 was intended to cause greater droplet spreading, reduce concentrations of products per unit leaf area, and also potentially improve uptake by stomatal infiltration on the abaxial surfaces.

Statistics

Analysis of variance carried out on the entire contact phytotoxicity set (Table 5) found the majority of interactions to be highly significant ($P< 0.001$). For this reason, the Mean Square values were looked at to ascertain which main effects and interactions were most important. The main effects with highest significance were found to be leaf surface, drop size, active ingredient concentration and time ($P< 0.001$). Species was shown to be not significant ($P> 0.1$). However all first order interactions with species were highly significant ($P< 0.001$), the most important first order interactions of species being with leaf surface and with drop size. Other important first order interactions were leaf surface with drop size, leaf surface with active ingredient concentration and leaf surface with time. The second order interaction, of species together with both leaf surface and active ingredient concentration, was also shown to be highly significant ($P < 0.001$).

Table 5: ANOVA table for complete contact phytotoxicity data

Analysis of Variance Procedure

Contact Phytotoxicity

Source	DF	Anova SS	Mean Square	F value	P
FORMULATION	10	26.30815567	2.63081557	4.98	0.0001
SPECIES	2	0.00783011	0.00391506	0.01	0.9926
FORM*SPECIES	20	105.92898257	5.29644913	10.03	0.0001
LEAF SURFACE	1	501.59369098	501.59369098	949.92	0.0001
FORM*SURFACE	10	29.144458153	2.91445815	5.52	0.0001
SPECIES*SURFACE	2	55.36134462	27.68067231	52.42	0.0001
FORM*SPECIES*SURFACE	20	34.16271132	1.70813557	3.23	0.0001
DROP SIZE	1	59.69280300	59.69280300	113.05	0.0001
FORM*SIZE	10	14.88014978	1.48801498	2.82	0.0019
SPECIES*SIZE	2	22.56543101	11.28271550	21.37	0.0001
FORM*SPECIES*SIZE	20	15.87841593	0.79392080	1.50	0.0724
SURFACE*SIZE	1	34.32544462	34.32544462	65.01	0.0001
FORM*SURFACE*SIZE	10	8.55063417	0.85506342	1.62	0.0965
SPECIES*SURFACE*SIZE	2	4.74150623	2.37075311	4.49	0.0115
FORM*SPECIES*SURFACE*SIZE	20	9.66191893	0.48309595	0.91	0.5680
CONCENTRATION	2	386.72976188	193.36488094	366.19	0.0001
FORM*CONC	20	54.61821074	2.73091054	5.17	0.0001
SPECIES*CONC	4	13.59280052	3.39820013	6.44	0.0001
FORM*SPECIES*CONC	40	38.36239817	0.95905995	1.82	0.0018
SURFACE*CONC	2	118.82156466	59.41078233	112.51	0.0001
FORM*SURFACE*CONC	20	36.09480953	1.80474048	3.42	0.0001
SPECIES*SURFACE*CONC	4	57.05046181	14.26261545	27.01	0.0001
FORM*SPECIES*SURFACE*CONC	40	32.14657396	0.80366435	1.52	0.0217
SIZE*CONC	2	1.89935865	0.94967932	1.80	0.1662
FORM*SIZE*CONC	20	12.08514665	0.60425733	1.14	0.2979
SPECIES*SIZE*CONC	4	12.20818350	3.05204587	5.78	0.0001
FORM*SPECIES*SIZE*CONC	40	15.72280163	0.39307004	0.74	0.8772
SURFACE*SIZE*CONC	2	0.93339735	0.46669867	0.88	0.4136
FORM*SURFACE*SIZE*CONC	20	12.04608490	0.60230424	1.14	0.3015
SPECIES*SURFACE*SIZE*CONC	4	1.42561453	0.35640363	0.67	0.6095
FORM*SPECIES*SURFACE*SIZE*CONC	39	19.30061577	0.49488758	0.94	0.5817
Between droplet residual	782	412.92600733	0.52803837	4.77	0.0001
TIME	4	410.23583496	102.55895874	926.09	0.0001
FORM*TIME	40	23.50213280	0.58755332	5.31	0.0001
SPECIES*TIME	8	36.53745710	4.56718214	41.24	0.0001
FORM*SPECIES*TIME	80	38.06881786	0.47586022	4.30	0.0001
SURFACE*TIME	4	53.93781305	13.48445326	121.76	0.0001
FORM*SURFACE*TIME	40	7.73615826	0.19340396	1.75	0.0026
SPECIES*SURFACE*TIME	8	1.40007086	0.17500886	1.58	0.1253
FORM*SPECIES*SURFACE*TIME	80	15.97514369	0.19968930	1.80	0.0001

TIME*SIZE	4	5.75266699	1.43816675	12.99	0.0001
FORM*TIME*SIZE	40	4.07349207	0.10183730	0.92	0.6156
SPECIES*TIME*SIZE	8	3.34108208	0.41763526	3.77	0.0002
FORM*SPECIES*TIME*SIZE	80	11.62641135	0.14533014	1.31	0.0342
SURFACE*TIME*SIZE	4	3.33928412	0.83482103	7.54	0.0001
FORM*SURFACE*TIME*SIZE	40	3.59719962	0.08992999	0.81	0.7940
SPECIES*SURFACE*TIME*SIZE	8	2.13435922	0.26679490	2.41	0.0137
FORM*SPECIES*SURFACE*TIME*SIZE	80	9.86340723	0.12329259	1.11	0.2320
TIME*CONC	8	61.45821542	7.68227693	69.37	0.0001
FORM*TIME*CONC	80	24.94348223	0.31179353	2.82	0.0001
SPECIES*TIME*CONC	16	14.53723201	0.90857700	8.20	0.0001
FORM*SPECIES*TIME*CONC	160	28.73153454	0.17957209	1.62	0.0001
SURFACE*TIME*CONC	8	10.06750414	1.25843802	11.36	0.0001
FORM*SURFACE*TIME*CONC	80	16.46608077	0.20582601	1.86	0.0001
SPECIES*SURFACE*TIME*CONC	16	3.38020059	0.21126254	1.91	0.0158
FORM*SPECIES*SURFACE*TIME*CONC	160	30.99916903	0.19374481	1.75	0.0001
TIME*SIZE*CONC	8	5.92569838	0.74071230	6.69	0.0001
FORM*TIME*SIZE*CONC	80	12.78875341	0.15985942	1.44	0.0065
SPECIES*TIME*SIZE*CONC	16	5.94323388	0.37145212	3.35	0.0001
FORM*SPECIES*TIME*SIZE*CONC	160	19.92091715	0.12450573	1.12	0.1414
SURFACE*TIME*SIZE*CONC	8	3.69492380	0.46186547	4.17	0.0001
FORM*SURFACE*TIME*SIZE*CONC	80	10.37832619	0.12972908	1.17	0.1438
SPECIES*SURFACE*TIME*SIZE*CONC	16	2.95497659	0.18468604	1.67	0.0458
TMT*SPECIES*SURFACE*TIME*SIZE*CONC	156	18.21442795	0.11675915	1.05	0.3105
Residual	3125	346.07399267	0.11074368		
Total	5881	3396.3673920			

FORM = Formulation; Species = Plant species; Surface = Leaf surface (adaxial or abaxial); Size = drop size (0.24 or 4 μl); Conc = concentration of triclopyr; Time = time at leaf was looked at with respect to contact phytotoxicity (2, 4, 6, 8 and 24 hours).

It was found that 4 μl droplets of the two organosilicone surfactants, Silwet L-77 and Silwet 408, both made up at 0.2% (w/v), did not cause any contact phytotoxicity over the 24 hour time period to any of the three tree species (Table 6). N-octyl pyrrolidone showed severe contact phytotoxicity towards both the adaxial and abaxial surfaces of sweetgum. When n-octyl pyrrolidone is an additive in a formulation this contact phytotoxicity doesn't stand out. An explanation for this is that in the formulations, an organosilicone surfactant is also added, enhancing spread, therefore lowering the concentration of n-octyl pyrrolidone per unit area. The alkylphenolic glycol ether was the least phytotoxic of the three wetter additives. The alcohol ethoxylate caused mild contact phytotoxicity to the abaxial leaf surfaces of all three tree species.

Table 6: Contact phytotoxicity (mean of observations made at 2, 4, 6, 8 and 24 hours) caused by 4.0 µl droplets of adjuvants alone onto adaxial (AD) and abaxial (AB) leaf surfaces of red maple, sweetgum and red oak.

	Conc.	Red Maple		Sweetgum		Red Oak		$\bar{x}$ treatments
	(%)	AD	AB	AD	AB	AD	AB	
Silwet 408	0.2	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{A}
Silwet L-77	0.2	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{A}
n-octyl pyrrolidone	0.1	0.53^{bc}	0.07^{a}	1.60^{d}	1.68^{d}	0.33^{ab}	0.73^{c}	0.82^{C}
alcohol ethoxylate	0.1	0.13^{ab}	0.4^{cd}	0.27^{bc}	0.53^{d}	0^{a}	0.4^{cd}	0.29^{B}
alkylphenolic glycol ether	0.1	0^{a}	0.13^{ab}	0.2^{b}	0.13^{ab}	0^{a}	0^{a}	0.08^{A}
$\bar{x}$ species x leaf surface		0.133^{AB}	0.12^{A}	0.413^{C}	0.467^{C}	0.067^{A}	0.227^{B}	

Within each row of the table, values followed by the same lowercase letter do not differ significantly (p=0.05). Comparisons among treatment averages, and among species x surface averages, are indicated using uppercase letters.

Red Maple, 0.24 µl.

With 0.32% a.e. (Figure 6 A, B), all formulations, except the base triclopyr amine formulation (3), caused nil or negligible contact phytotoxicity to both adaxial and abaxial leaf surfaces of red maple. A possible explanation for the base triclopyr amine formulation (TTEA) causing higher contact phytotoxicity to both leaf surfaces is that it contains no surfactant to aid spreading and therefore there is a higher concentration of active ingredient per unit area. At 1.6% a.e (Figure 6 C, D) there is nil or negligible contact phytotoxicity to the adaxial surface by all formulations, except again the TTEA formulation. The TTEA formulation (3) and Garlon 3A (2) show severe phytotoxicity on the abaxial surface at 24 and 8 hours respectively (maximum rating of 2) while Garlon 4 (1) and TTEA +Silwet L-77 (11) show high contact phytotoxicity at 24 hours. At 3.2% a.e. (Figure 6 E, F) there is nil to mild contact phytotoxicity to the adaxial surface by all formulations except for the TTEA formulation (3), Garlon 3A (2) and the TTEA plus alcohol ethoxylate plus Silwet 408 formulation (6). The TTEA plus alcohol ethoxylate plus Silwet 408 formulation (6) shows the earliest contact phytotoxicity towards the abaxial leaf surface, and

causes extreme phytotoxicity within 6 hours. The formulation causing least contact phytotoxicity is TTEA plus alkylphenolic glycol ether plus Silwet 408 (8), followed by Garlon 4 (1). Comparing the adaxial surface versus the abaxial surface in Figure 6, it can be seen that contact phytotoxicity to the adaxial leaf surface is nearly always less than or equal to contact phytotoxicity caused to the abaxial leaf surface. It can be seen readily from Figure 6 that increasing ai concentration causes an increase in contact phytotoxicity. An example of contact phytotoxicity caused by 0.24 µl drops on red maple can be seen in Figure 7, a and b.

Red Maple, 4 µl.

Comparing Figure 6 and Figure 8 it can readily be seen that increasing the droplet size increases contact phytotoxicity ($P<0.001$). Contact phytotoxicity results could not be obtained for 0.32% a.e. TTEA on the abaxial leaf surface of red maple as the droplets would not adhere to the surface. Garlon 4 (1) causes severe contact phytotoxicity to the abaxial leaf surface after 6 hours (Figure 8). At 1.6% a.e. TTEA plus Silwet L-77 (11) causes severe contact phytotoxicity to the adaxial leaf surface at 24 hours. Garlon 4 (1) causes severe contact phytotoxicity to the abaxial surface by 4 hours. Contact phytotoxicity to the abaxial surface is high to severe for all formulations by 24 hours. At 3.2% a.e. TTEA formulation (3) and TTEA formulation plus alcohol ethoxylate plus Silwet L-77 (7) show severe contact phytotoxicity to the adaxial surface at 24 hours. Severe contact phytotoxicity is caused to the abaxial leaf surface by all formulations. Again it can be seen (Figure 8) that contact phytotoxicity to the adaxial leaf surface is less than or equal to contact phytotoxicity to the abaxial leaf surface and that increasing concentration of active ingredient caused increased contact phytotoxicity.
Examples of contact phytotoxicity caused by 4 µl droplets can be seen in Figure 7, c, d and e.

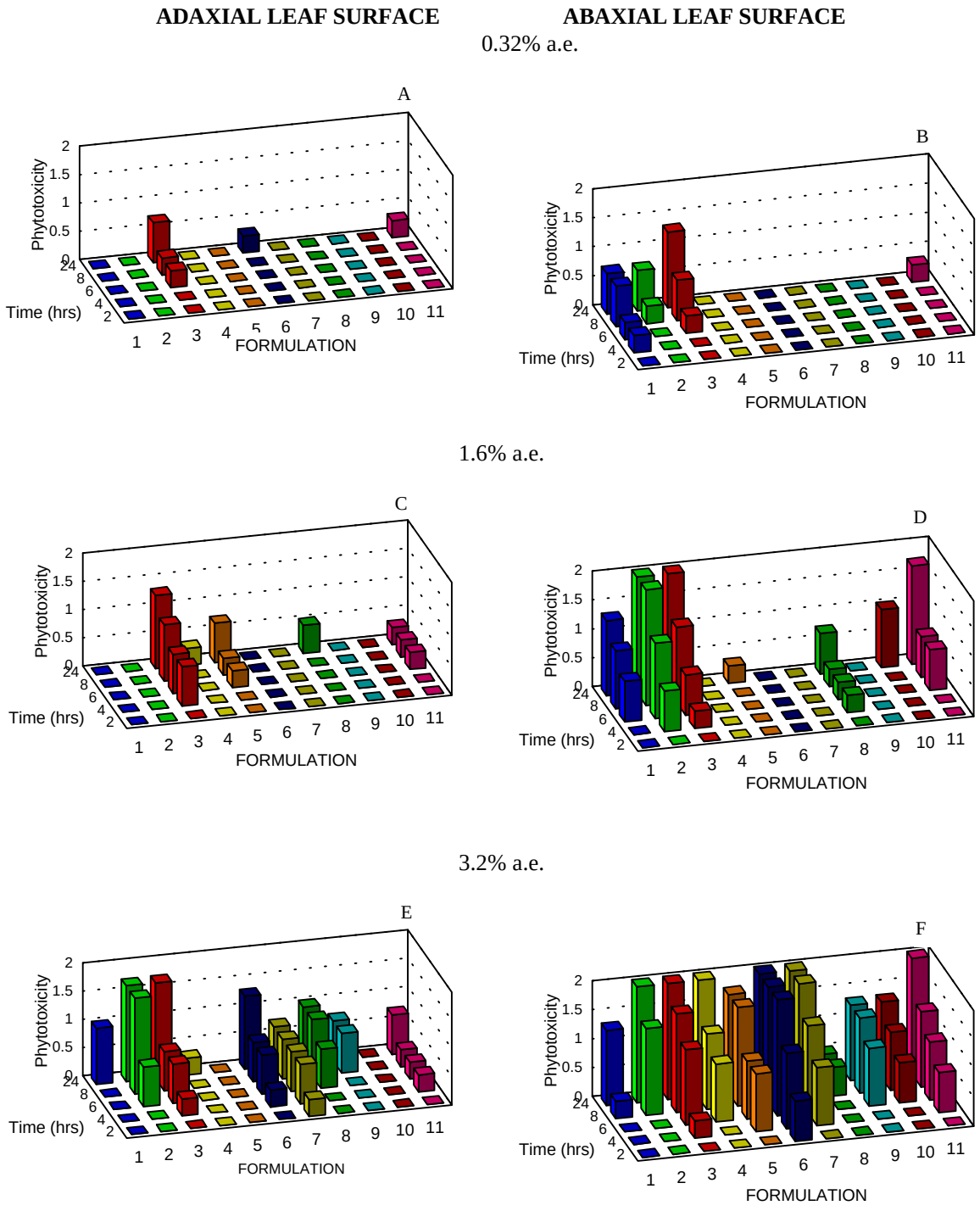

Figure 6: Comparison of contact phytotoxicity caused by 0.24 ul droplets of 11 formulations at 3 concentrations to adaxial and abaxial surfaces of red maple.

Formulations: 1 = Garlon 4, 2 = Garlon 3A, 3 = triclopyr TEA + sequestrant (TTEA), 4 = TTEA + n-octyl pyrrolidone + Silwet 408, 5 = TTEA + n-octyl pyrrolidone + Silwet L-77, 6 = TTEA + alcohol ethoxylate + Silwet 408, 7 = TTEA + alcohol ethoxylate + Silwet L-77, 8 = TTEA + alkylphenolic glycol ether + Silwet 408, 9 = TTEA + alkylphenolic glycol ether + Silwet L-77, 10 = TTEA + Silwet 408, 11 = TTEA + Silwet L-77

a. *10% Garlon 3A applied as 0.24 ul droplets to the abaxial surface (adaxial view).*

b. *10% Garlon 3A applied as 0.24 ul droplets to the abaxial surface.*

c. *10% triclopyr TEA + sequestrant applied as 4 ul droplets to the adaxial surface.*

d. *10% Garlon 3A applied as 4 ul droplets to the abaxial surface (adaxial view).*

e. *10% Garlon 3A applied as 4 ul droplets to the abaxial surface.*

Figure 7: Examples of contact phytotoxicity on red maple.

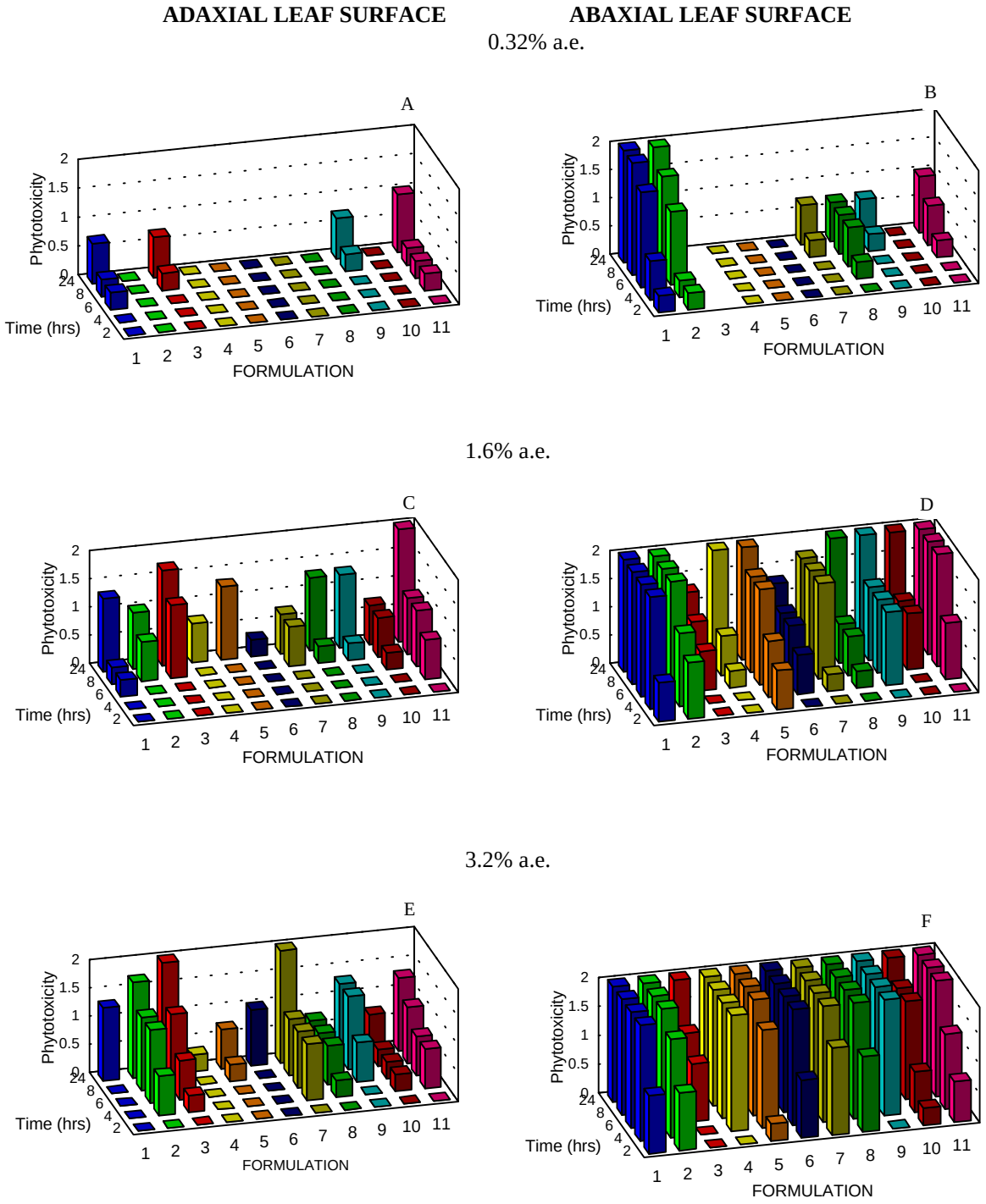

Figure 8: Comparison of contact phytotoxicity caused by 4 ul droplets of 11 formulations at 3 concentrations to adaxial and abaxial surfaces of red maple.

Formulations: 1 = Garlon 4, 2 = Garlon 3A, 3 = triclopyr TEA + sequestrant (TTEA), 4 = TTEA + n-octyl pyrrolidone + Silwet 408, 5 = TTEA + n-octyl pyrrolidone + Silwet L-77, 6 = TTEA + alcohol ethoxylate + Silwet 408, 7 = TTEA + alcohol ethoxylate + Silwet L-77, 8 = TTEA + alkylphenolic glycol ether + Silwet 408, 9 = TTEA + alkylphenolic glycol ether + Silwet L-77, 10 = TTEA + Silwet 408, 11 = TTEA + Silwet L-77