Sterilizing Effects of Diflubenzuron and its Analogs in the Azuki Bean Weevil, *Callosobruchus chinensis* L. (Coleoptera, Bruchidae)

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Diflubenzuron およびその同族体のアズキゾウムシに対する不妊作用

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Diflubenzuron (*N*-[[(4-chlorophenyl) amino] carbonyl]-2, 6-difluorobenzamide, AI3-29054) and some of its analogs induce sexual sterility in both sexes of the boll weevil, *Anthonomus grandis* Boheman.[1] Treated females crossed with untreated males oviposited normally, but their eggs did not hatch. When treated males were crossed with untreated females the results were similar, however, the sterility resulted from a transfer of the compound from males to females during copulation and confinement. Apparently, the chitin-synthesis inhibiting effects of these sterilants[2] interfered with embryogenesis, and the compounds had to reach the female directly by treatment or indirectly by transfer from a treated male. This unusual mode of action prompted us to investigate the effects of diflubenzuron and 35 of its analogs in the azuki bean weevil, *Callosobruchus chinensis* L., and the results are reported here.

Materials and Methods

Compounds. - Diflubenzuron and 34 of its analogs were synthesized in the Insect Chemosterilants Laboratory and their purity was 99-100%. A sample of AI3-29368 was kindly donated to us by Chemagro Agricultural Chemicals Division, Mobay Chemical Corp., Kansas City, MO, U. S. A. Of the 36 compounds tested, 28 were inactive; the AI3-numbers and names of the 8 active compounds follow:

AI3-29053 2, 6-dichloro-*N*-[[(4-chlorophenyl) amino]carbonyl] benzamide
AI3-29054 *N*-[[(4-chlorophenyl) amino]carbonyl]-2, 6-difluorobenzamide; diflubenzuron
AI3-29368 2-chloro-*N*-[[[4-(trifluoromethoxy)phenyl]amino] carbonyl]benzamide
AI3-63061 *N*-(4-chlorophenyl)-*N*'-(2, 6-difluorobenzoyl)thiourea
AI3-63218 *N*-[[(3, 4-dichlorophenyl)amino]carbonyl]-2, 6-difluorobenzamide
AI3-63220 *N*-[[(4-bromophenyl)amino]carbonyl]-2, 6-difluorobenzamide
AI3-63223 2, 6-difluoro-*N*-[[[4- (trifluoromethyl) phenyl] amino] carbonyl] benzamide; penfluron

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Table 1. —Oviposition of production of adult progeny by azuki bean weevils treated with 1 μg/insect of diflubenzuron (AI3-29054) or its analogs. (Averages of 10 replicates).

Compound	Sex treated					
	Female		Mael		Both	
	No. eggs deposited	No. adults emerged	No. eggs deposited	No. adults emerged	No. eggs deposited	No. adults emerged
AI3-63223	70.6	0	66.8	6.8	69.8	1.3
AI3-63220	65.4	1.1	68.9	13.0	64.3	1.7
AI3-29368	68.7	1.7	73.8	70.2	69.8	67.8
AI3-63386	69.0	2.0	70.2	7.2	67.8	3.2
AI3-29054	71.6	2.7	63.3	16.7	65.5	1.4
AI3-63218 1/	56.9	6.0	77.3	48.9	60.1	6.8
AI3-29053	74.1	8.8	76.6	56.4	78.8	9.1
AI3-63061	30.0	14.0	64.8	57.7	39.7	11.2
Untreated controls	77.9	67.2				

1/The dose was 0.5 μg/weevil

AI3-63386 2, 6-difluoro-*N*-[[[3-(trifluoromethyl)phenyl]amino] carbonyl]benzamide

Since all the diflubenzuron analogs were partially soluble in acetone, the latter was selected as the most suitable solvent. However, the solubility of AI3-63218 was so low that the standard does of 1 $\mu g/mm^3$ of acetone could not be reached and the compound was tested at 0.5 $\mu g/mm^3$.

Insects. - The azuki bean weevils used as the test organisms were the progeny of a strain inbred at the Chemical Contamination Biology Laboratory, Faculty of Agriculture, Shimane University, for several years. The water content of the azuki beans was about 15.3 percent. Adult weevils, just emerged from beans, were used for tests.

Test methods. - One mm^3 of acetone containing appropriate concentrations of the test compounds was applied to the dorsal side of each weevil by a micropipette. Each pair of weevils consisting of a treated female and untreated male, untreated female and treated male, or treated male and female, was confined with ca. 70 azuki beans in a covered polyethylene cup having a diameter of 6.5 cm and a height of 3.5 cm. Deposited eggs were counted 15-20 days posttreatment and 15-25 days later the emerged adults were scored. Experiments were repeated 5-10 times for each dose level and combination and controls were treated with acetone only. Rearing and all experiments were conducted in a lighted laboratory at 25°C and 50% R. H.

In competitive mating experiments, males were treated with 0.015625-1.0 μg of diflubenzuron and confined in various ratios with untreated males and females in groups of 20 in a covered polyethylene cup (diam. 10 cm, height 4.5 cm) containing ca. 200 azuki beans. The fecundity and fertility of the females were determined in the same way as described earlier. Each experiment was replicated 5 times.

Results and Discussion

Sterilizing activity of urea derivatives. -Initial studies of Wellinga et al.[3,4] have shown that certain substituted *N*-benzoyl-*N'*-phenylureas were toxic to several species of insects. Later work[5,6,7] indicated that these and related materials have also insect sterilizing properties, but

Table 2. —Numbers of eggs deposited and adult progeny produced by single pairs of azuki bean weevils treated with various doses of diflubenzuron. Averages of 5 replicates.

Sex treated	Dose (μg/weevil)	No. eggs deposited	No. adults emerged
Female	0.7×0.5^3	64.0	11.0
	1×0.5^4	69.8	10.0
	0.7×0.5^4	67.8	12.0
	1×0.5^5	64.4	12.2
	0.7×0.5^5	64.5	21.5
	1×0.5^6	62.4	30.2
	0.7×0.5^6	75.6	48.8
	1×0.5^7	80.2	51.8
	0.7×0.5^7	64.4	48.0
	1×0.5^8	65.0	52.5
	0.7×0.5^8	64.2	55.6
	1×0.5^9	80.5	73.8
Male	1	76.6	20.4
	0.7	67.3	19.3
	1×0.5^1	74.0	29.2
	0.7×0.5^1	76.8	37.5
	1×0.5^2	73.4	39.0
	0.7×0.5^2	74.2	51.6
	1×0.5^3	67.8	50.6
	0.7×0.5^3	72.4	54.2
	1×0.5^4	78.4	66.8
	0.7×0.5^4	74.0	68.8
Untreated controls		66.6	60.8

Table 3. —Analysis of χ^2 for diflubenzuron-treated azuki bean weevils.

Source of variation	D.F.	Sum of squares	Mean square
Parallelism of regression	1	1.04	1.04
Residual heterogeneity	18	15.64	0.87
Total	19	16.68	—

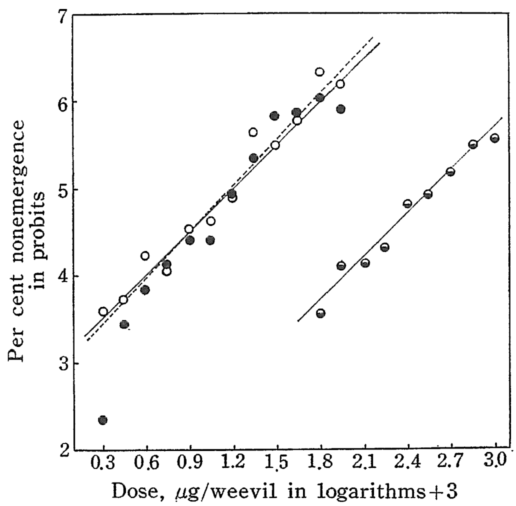

Fig. 1. Dose-nonemergence relations for azuki bean weevils treated with diflubenzuron. ● ...T♀×U♂, ◒...U♀×T♂, ○...T♀×T♂.

except some broad generalizations, no distinct relationship between their chemical structure and sterilizing activity could be demonstrated. The present study with 36 urea and thiourea derivatives applied topically to azuki bean weevils confirms this conclusion. The list of active compounds in Table 1 shows that the most effective substituents in the benzoyl moiety of the urea derivatives were fluorine atoms in the 2- and 6- positions ; chlorine atoms in the same positions were also effective. In the phenyl moiety, trifluoromethyl, bromo, trifluoromethoxy, and chloro groups in the 4- and 3-positions were effective. Compared with analogous ureas, the corresponding thioureas were less active sterilants. As in the boll weevil[8] the most effective sterilant in the azuki bean weevil was penfluron (AI3-63223), but because the closely related and highly active diflubenzuron (AI3-29054) is in commercial development, our more detailed investigation centered on this latter material.

Differential susceptibility of sexes to diflubenzuron. - Results in Table 2 show conclusively that females were more susceptible than males to this compound. Since on the average, 60.8 adults emerged from 66.6 control eggs, the mortality during adult emergence in controls (c) was 8.7%. At the lowest dose level, mortality was 8.3 and 7.0% in female and male treatments, respectively, and the provisional value of $C = 8.0\%$ was assigned. Following the maximum likelihood solution adjusted for natural mortality,[9] parameters of the tolerance distribution between the log dose and probit percent nonemergence the first level revised estimate of $C = 6.3\%$. Because of the closeness of these two estimates, no further calculation levels were required. The analysis of χ^2 in Table 3 shows that the

Table 4. —Presumption of dose-response rates in diflubenzuron-treated female × male combinations.

Dose (μg/weevil)	Number of eggs	Number of adults	Observed response rates	Expected response rates
0.7×0.5^3	68.0	7.4	0.882	0.907
1×0.5^4	65.6	5.6	0.908	0.857
0.7×0.5^4	62.0	12.4	0.783	0.781
1×0.5^5	62.3	17.7	0.691	0.696
0.7×0.5^5	50.5	12.0	0.741	0.600
1×0.5^6	57.6	28.8	0.457	0.498
0.7×0.5^6	51.0	30.5	0.350	0.400
1×0.5^7	79.0	49.5	0.318	0.307
0.7×0.5^7	72.0	54.3	0.180	0.225
1×0.5^8	77.8	55.8	0.220	—
0.7×0.5^8	56.0	46.3	0.101	—
1×0.5^9	76.4	64.6	0.080	—

components for deviation from parallelism and for residual heterogeneity are small and can be attributed to random variation of the data. The calculated dose-nonemergence regression equation for treated female × untreated male (T♀×U♂) was $Y=3.0102+1.6587x$, and that for untreated female × treated male (U♀×T♂) was $Y=0.7386+1.6587x$; Fig. 1 shows the calculated and experimental regression lines with $x=\log\mu g+3$. The most probable median sterilizing dose in T♀×U♂ experiments was 0.0158 μg/♀, with fiducial limits 0.0196 and 0.0128 μg/♀ for 95% probability, and in U♀×T♂ experiments 0.3708 μg/♂, limits 0.3939 and 0.3490 μg/♂ : Consequently, the female was 23.42 times (limits 29.66−18.49) more susceptible to diflubenzuron than the male. These results are in agreement with the differential susceptibility of male and female boll weevils to diflubenzuron and penfluron.[8]

Presumption of joint action. — Nagasawa and Nakayama[10] and Nagasawa[6] have shown that when both sexes of an insect were susceptible to a chemosterilant, the results of crossing treated females with treated males (T♀×T♂) can be simulated by a mathematical model of independent joint action with a correlation $r=0$. The expected response rate P for mating (T♀×T♂) should then be

$$P=P_{♀}+P_{♂}(1-P_{♀}),$$

where $P_{♀}$ and $P_{♂}$ are the response rates induced from the same amount of sterilant used in T♀×U♂ and U♀×T♂, respectively.

The numbers of deposited eggs and of emerged adults from T♀×T♂ at different dose levels are shown in Table 4. As before, the emergence rates were corrected by Abbott's formula with $C=8.0$ (fourth column). The regression lines (Fig. 1) and their corresponding equations were then used to calculate the expected response rate from independent joint action and the results appear in the last column in Table 4. A test of agreement between the hypothesis and observation was made by finding the weight to be associated with the expected probits corresponding to expected response rates, multiplying these by the squares of the corresponding discrepancy between expected and observed probits, and summing to give χ^2 with degrees of freedom equal to the number of dose levels. Since $\chi^2_{[9]}=9.108$, there was no evidence of conflict with the hypothesis, and the dose-response data from T♀×T♂ can be presumed from similar data obtained from T♀×U♂ and U♀×T♂ by the mathematical model of independent joint action with a correlation $r=0$.

Table 5. —Mating competitiveness of diflubenzuron-treated and untreated azuki bean weevils. Oviposition and adult emergence at different mating ratios T♂:U♂:U♀.

Treatment dose (μg/♂)	Mating ratio T♂ : U♂ : U♀	No. eggs deposited						No. adults emerged					
		I	II	III	IV	V	Total	I	II	III	IV	V	Total
1	0:10:10:	608	736	667	688	684	3383	331	452	431	492	621	2327
	1: 9:10:	518	613	881	655	631	3298	247	344	380	369	237	1577
	2: 8:10:	562	470	583	714	692	3021	199	351	200	459	163	1372
	3: 7:10:	795	546	664	779	612	3396	137	97	156	270	91	751
	4: 6:10:	361	718	635	723	627	3064	89	136	95	111	98	529
	5: 5:10:	730	683	551	665	572	3201	148	126	89	127	86	576
	6: 4:10:	470	488	530	661	551	2700	72	97	66	91	117	443
	7: 3:10:	479	590	773	793	553	3188	70	95	99	115	85	464
	8: 2:10:	613	788	499	788	594	3282	59	80	89	106	109	443
	9: 1:10:	608	607	478	682	625	3000	56	103	54	83	74	370
	10: 0:10:	692	840	512	631	756	3431	55	87	59	93	62	356
	Total	6436	7079	6773	7779	6897	34964	1463	1968	1718	2316	1743	9208
0.25	0:10:10:	725	574	724	604	751	3378	416	396	464	626	648	2550
	1: 9:10:	768	610	781	655	715	3529	384	421	427	591	646	2469
	2: 8:10:	707	556	630	684	620	3197	403	365	411	624	502	2305
	3: 7:10:	736	745	602	677	630	3390	278	291	410	613	477	2069
	4: 6:10:	729	611	610	675	618	3243	172	354	231	534	265	1556
	5: 5:10:	647	535	485	661	633	2961	131	183	271	523	180	1288
	6: 4:10:	625	732	556	682	613	3208	454	144	209	552	191	1550
	7: 3:10:	670	621	542	598	605	3036	140	269	142	249	92	892
	8: 2:10:	654	750	625	676	607	3312	135	161	112	539	98	1045
	9: 1:10:	623	706	579	716	615	3239	119	125	122	148	89	603
	10: 0:10:	701	679	565	843	681	3469	132	146	103	201	90	672
	Total	7585	7119	6699	7471	7088	35962	2764	2855	2902	5200	3278	16999
0.0625	0:10:10:	619	696	581	759	731	3386	386	436	421	681	624	2548
	1: 9:10:	572	694	615	770	557	3208	369	459	475	627	577	2507
	2: 8:10:	568	663	536	745	761	3273	422	425	460	619	604	2530
	3: 7:10:	655	819	589	803	621	3487	443	466	484	573	546	2512
	4: 6:10:	619	766	547	707	742	3381	421	521	488	681	617	2728
	5: 5:10:	702	604	598	772	785	3461	436	433	442	612	649	2572
	6: 4:10:	732	607	596	642	707	3284	435	396	454	512	580	2377
	7: 3:10:	681	705	583	728	729	3426	366	466	319	637	600	2388
	8: 2:10:	682	593	605	724	719	3323	383	277	543	627	469	2299
	9: 1:10:	709	590	547	726	769	3341	440	114	450	620	630	2254
	10: 0:10:	750	730	601	712	663	3456	438	468	461	575	515	2457
	Total	7289	7467	6398	8088	7784	37026	4539	4461	4997	6764	6411	27172
0.015625	0:10:10:	681	606	584	726	758	3355	619	342	507	678	623	2769
	1: 9:10:	628	589	772	729	693	3411	447	432	569	626	612	2686
	2: 8:10:	655	611	641	623	648	3178	520	439	574	587	538	2658
	3: 7:10:	703	569	587	676	743	3278	504	393	511	638	630	2676
	4: 6:10:	708	448	795	703	658	3312	408	366	580	681	566	2601
	5: 5:10:	743	575	708	703	625	3354	425	484	525	628	538	2600
	6: 4:10:	658	681	685	690	790	3504	498	445	383	632	657	2615
	7: 3:10:	667	680	700	758	588	3393	499	392	583	588	517	2579
	8: 2:10:	664	631	701	708	701	3405	446	260	463	649	546	2364
	9: 1:10:	689	619	657	757	660	3382	400	295	515	617	599	2426
	10: 0:10:	624	601	599	788	597	3209	452	456	428	701	590	2627
	Total	7420	6610	7429	7861	7461	36781	5218	4304	5638	7025	6416	28601

Mating competitiveness of treated and untreated males. —The present experimental design has been used previously by Nagasawa and Shinohara[11] who used the total number of eggs deposited as a measure of mating competitiveness and analyzed the data statistically by analysis of variance. They concluded that the mating ability of apholate-treated male azuki bean weevils was unimpaired because the ratio of variance for the combination T♂ : U♂ : U♀ to that for the error was not significant. Nagasawa et al.[12] used another method

Table 6. —Analysis of variance for data in Table 5.

Dose μg/♂	Term	DF	Number of eggs deposited			Number of adults emerged		
			SS	MS	F	SS	MS	F
1	Combination	10	95442.55	9544.26	0.88	824671.38	206167.85	67.66*
	Replication	4	90227.90	2256.98	0.21	37215.38	3721.54	1.22
	Error	40	435666.90	10891.67		121880.62	3047.02	
	Total	54	621337.35			983767.38		
0.25	Combination	10	59120.84	5912.08	1.47	1021614.51	102162.45	12.08*
	Replication	4	44680.29	11170.07	2.78*	373202.62	93300.66	11.03*
	Error	40	160527.71	4013.19		338254.58	8456.36	
	Total	54	264328.84			1733071.71		
0.0625	Combination	10	15760.40	1576.04	0.42	36244.73	3624.47	0.78
	Replication	4	149225.35	37306.34	9.84*	423831.93	105957.98	22.71*
	Error	40	151727.05	3793.18		186659.27	4666.48	
	Total	54	316712.80			646735.93		
0.015625	Combination	10	17807.24	1780.72	0.49	26492.98	2649.30	0.76
	Replication	4	75635.53	18908.88	5.24*	404656.80	101164.20	29.10*
	Error	40	146645.67	3666.14		139067.20	3476.68	
	Total	54	240088.44			570216.98		

of analysis for similar experimental data. For a simple test, weighting each number of deposited eggs equally, the trend and the curvature with orthogonal polynominals x_1 and x_2 were checked, the scatter about the fitted line being used as the error. The lack of difference among the numbers of eggs deposited in each combination was then used to prove that triphenyltin hydroxide-treated males were fully competitive with untreated males in mating with untreated females.

The data from the present experiment are summarized in Table 5 and their analysis of variance is presented in Table 6. With regard to oviposition, there was no significant variation in the number of eggs at different T♂ : U♂ ratios or at different dose levels. The data are presented graphically in Fig. 2. These results indicate that the mating ability of diflubenzuron-treated males was the same as that of untreated males. On the other hand, as shown graphically in Fig. 3, the number of emerged adults decreased with the increase in T♂ : U♂, most dramatically at the two higher dose levels. Analysis of variance has shown that although the emergence differences at the two lower levels were not significant, those at the higher levels were clearly significant.

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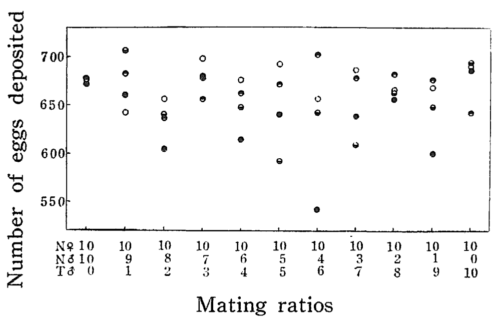

Fig. 2. Vaiatiorn in oviposition resulting from different mating ratios of azuki bean weevils (T♂:U♂:U♀).

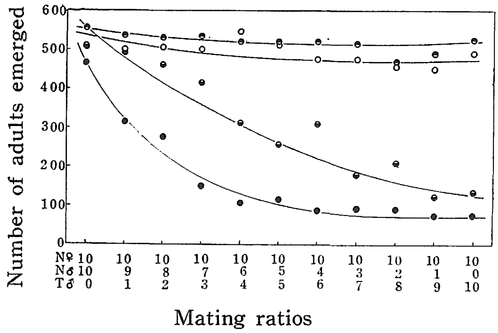

Fig. 3. Variation in production of adult progeny from different mating ratios of azuki bean weevils (T♂:U♂:U♀).

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摘　　要

Urea および thiourea 系化合物36試料のアズキゾウムシに対する，不妊作用を微量滴下法によって検定した結果，diflubenzuron を含む 8 試料に，顕著な不妊性がみとめられた．雌は diflubenzuron に対して，雄の約23倍の感受性を示した．雌雄の両方に diflubenzuron を処理して，それらの交配によって得られる薬量—反応率曲線は，雌，雄いずれか一方に処理してえられたふたつの薬量—反応率曲線から，相関 $r=0$ とする independent joint action の数学的モデルによって推定できた．diflubenzuron を処理した雄は，無処理の雄と雌を獲得する性競合においてかわりない行動を示した．