Specificity of Pyridinemonocarboxylates and Benzoic Acid Analogues as Chemical Hybridizing Agents in Wheat

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A series of substituted pyridinemonocarboxylates and benzoic acids were evaluated in growth chambers as potential chemical hybridizing agents for wheat (*Triticum aestivum L.*). Chemical hybridizing potential, measured as spike sterility, was observed with both areas of chemistry. The 3-pyridinecarboxylic acid, 4-hydroxy-2,6-bis(trifluoromethyl) methyl ester, and 2,4-bis(trifluoromethyl)benzoic acid were the only molecules to exhibit complete spike sterility. Minor changes in both molecules resulted in total loss of activity. Substitutions at the 4-position on the pyridinemonocarboxylate which are subject to hydrolysis to the 4-hydroxyl or which contained an acidic proton functionality were the only substitutions exhibiting any level of spike sterility.

INTRODUCTION

Wheat (*Triticum aestivum* L.), a self-pollinated crop, is one of the major food crops in the world. To obtain major improvements in grain yield and/or disease resistance through hybrid vigor (heterosis), widely diverse germplasma needs to be crossed in the formation of wheat hybrids. In the production of hybrid wheat seed, either anther removal or pollen sterility is required in the female line to obtain the desired outcrossing. Pollen sterility can be achieved through genetic development of cytoplasmic male-sterile lines or the use of a chemical hybridizing agent (CHA). Several areas of chemistry have been reported to produce male sterility in wheat.

DPX 3778 [3-(*p*-chlorophenyl)-6-methoxy-*s*-triazine-2,4(1*H*,3*H*)-dione–triethanolamine] has shown control of pollen sterility in wheat (Johnson and Brown, 1978), oats (*Avena sativa* L.) (Johnson and Brown, 1976), and pearl millet (*Pennisetum americanum* (L.) Leeke) (Hanna, 1977). Hybridization of soft white winter wheat using ethephon (2-chloroethylphosphonic acid) has been reported (Rowell and Miller, 1974). More recently, LY195259 [5-(aminocarbonyl)-1-(3-methylphenyl)-1*H*-pyrazole-4-carboxylic acid] was reported to exhibit at least 90% male sterility in over 100 cultivars of wheat (Tschabold et al., 1988).

Pyridines and substituted benzoic acids are well-known for their herbicidal activity. Benzoic acid (Kobayashi et al., 1981) and substituted benzoic acids (Ejiri et al., 1984) have also been shown to suppress pollen germination in *Pinus densiflora*. This paper describes the specificity of pyridinemonocarboxylates and substituted benzoic acids to produce pollen sterility in wheat.

MATERIALS AND METHODS

A series of pyridinemonocarboxylate and benzoic acid analogues (Tables I–III) varying in the chemical constituents on the respective pyridine and benzoic rings were synthesized to determine their chemical hybridizing potential in wheat. A number of benzoic acid analogues and other substituted phenyl rings were also obtained from Aldrich Chemical Co. (Milwaukee, WI) for evaluation.

Wheat plants were grown in growth rooms set at 19 °C/17 °C (day/night) temperatures with a 16-h photoperiod, a light intensity of approximately 800 μE, and 70% relative humidity. Seven seeds of the awned hard red spring wheat cultivar Anza were planted 2.5–4.0 cm deep in a 15.3-cm plastic pot containing a soilless medium of Metro-Mix 200 supplemented with 93 g of Osmocote (14-14-14) control release fertilizer, 93 g of Peters (14-7-7) slow release fertilizer, and 17 g of Micromax micronutrients per cubic foot of Metro-Mix 200. Approximately 10–14 days after planting, the pots were thinned to six plants per pot. Plants were maintained at the above environmental conditions throughout the experiment. Water was applied to each pot through an automatic watering system with the quantity applied being a function of the plant's age and development.

Chemical treatments were applied on the basis of the developmental stage of the spike identified from initial tests as having the maximum chemical hybridizing activity for the pyridinemonocarboxylates. For wheat, maximum activity occurred at a spike length of approximately 5 cm. Timing for chemical applications was determined by measuring the length of four to five spikes dissected from the largest tillers within a chamber. Chemicals were formulated in either water or acetone/water (50:50) depending on the solubility in water of the respective compounds with the addition of 0.2% Tween 20 (Universal Flavoring Corp., Indianapolis, IN) as a surfactant. Chemicals were applied to the plants in three pots using a spray volume of 48 mL (approximately 2800 L/ha) in an enclosed track sprayer chamber consisting of a compressed air reservoir (170 kPa) containing a TeeJet 400E spray nozzle and equipped with a manual speed adjustment. The spray nozzle was adjusted to a height of approximately 30 cm above and centered over the plants.

Groups of 8–12 compounds were examined at a time with an internal standard (Table I, example 3) included in all tests. Spray rates varied from test to test; however, three rates of application were used in the majority of all tests. Chemical rates consisted of a low (2.5–5.6 kg/ha), mid (8–11.2 kg/ha), and high (13.5–16.8 kg/ha) rate.

Glassine bags were placed over 12–15 spikes per pot to determine the level to sterility. Glassine bags were placed over the spikes and stapled after head emergence but prior to anthesis (anther extrusion) to prevent all but self-pollination from occurring within a spike. Approximately 4 weeks later, after the

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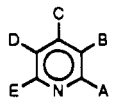
Table I. Growth Chamber POE Evaluation of Substituted Pyridinemonocarboxylates for Spike Sterility in Wheat at Three Application Rates

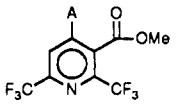


example	A	B	C	D	E	low[a]	mid	high
1	CF_3	H	O(IPA)[c]	H	CF_3	0[b]	0	0
2	CF_3	C(O)OMe	H	H	CF_3	0	0	0
3	CF_3	C(O)OMe	OH	H	CF_3	3	4	4
4	CF_2H	C(O)OMe	OH	H	CF_2H	0	0	0
5	CF_3	C(O)OMe	O(IPA)	H	CF_3	4	4	4
6	CF_3	C(O)OEt	OH	H	CF_3	0	0	0
7	CF_3	C(O)OEt	O(IPA)	H	CF_3	0	0	0
8	CF_3	C(O)OPr	OH	H	CF_3	0	0	0
9	CF_3	C(O)OPr	O(IPA)	H	CF_3	0	0	0
10	CF_3	$C(O)OCHMe_2$	OH	H	CF_3	0	0	0
11	CF_3	$C(O)OCHMe_2$	O(IPA)	H	CF_3	0	0	0
12	CF_3	$C(O)OCH_2(C_6H_5)$	OH	H	CF_3	0	0	0
13	CF_3	$C(O)OCH_2(C_6H_5)$	O(IPA)	H	CF_3	0	0	0
14	CF_3	$C(O)NH_2$	OH	H	CF_3	0	0	0
15	CF_3	$C(O)NH_2$	O(IPA)	H	CF_3	0	0	0
16	CF_3	COOH	OH	H	CF_3	0	0	0
17	CF_3	COO(IPA)	OH	H	CF_3	0	0	0
18	CF_3	COOH	H	H	CF_3	2	4	4
19	CF_3	COO(IPA)	H	H	CF_3	1	4	4
20	CF_3	C(O)OMe	OH	H	C(O)OMe	0	0	0
21	CF_3	C(O)OMe	OH	H	OH	0	0	-
22	Me	C(O)OMe	OH	H	Me	0	0	0
23	Me	C(O)OMe	O(IPA)	H	Me	0	0	0
24	Me	C(O)OEt	Et	COOH	Me	0	0	0
25	CF_3	C(O)OMe	O(IPA)	H	CF_2Cl	0	0	0
26	CF_3	C(O)OMe	O(IPA)	H	CF_2H	0	0	0
27	CF_2Cl	C(O)OMe	O(IPA)	H	CF_2Cl	0	0	0
28	CF_3	C(O)OEt	OH	H	CF_2Cl	0	0	0
29	CF_3	C(O)OEt	OH	Me	CF_2CF_3	0	0	0
30	CF_3	C(O)OEt	OMe	H	CF_3	0	0	0
31	CF_3	C(O)OMe	OH	C(O)OMe	CF_3	0	0	0
32	CF_3	C(O)OMe	O(IPA)	C(O)OMe	CF_3	0	0	0
33	CF_3	C(O)OMe	OH	OMe	CF_3	0	0	0
34	CF_3	C(O)OEt	OH	Me	CF_2H	0	0	0
35	CF_3	C(O)OMe	O(IPA)	OMe	CF_3	0	0	0
36	CF_3	C(O)OMe	O(IPA)	Me	CF_3	0	0	0
37	CF_3	C(O)OMe	OH	COOH	CF_3	0	0	0
38	CF_3	C(O)OMe	OH	COO(IPA)	CF_3	0	0	0
39	CF_3	C(O)OMe	O(IPA)	COO(IPA)	CF_3	0	0	0
40	CF_3	C(O)OMe	OH	$COOCMe_3$	CF_3	0	0	0
41	CF_3	C(O)OMe	OH	C(O)OMe	$CHMe_2$	0	0	0
42	CF_3	C(O)OMe	O(IPA)	C(O)OMe	$CHMe_2$	0	0	0
43	CF_3	C(O)OEt	O(IPA)	Me	CF_3	0	0	0
44	CF_3	C(O)OMe	OH	H	OH	0	0	-
45	CF_3	C(O)OEt	OH	H	OH	0	0	0
46	CF_3	C(O)OEt	O(IPA)	H	OH	0	0	0
47	CF_3	C(O)OEt	$OC(O)(C_6H_5)$	Me	CF_3	0	0	0
48	CF_2CF_3	C(O)OEt	O(IPA)	H	OH	0	0	0
49	CF_3	C(O)OEt	O(IPA)	H	CF_2Cl	0	0	0
51	CF_3	C(O)OEt	O(IPA)	H	CF_2H	0	0	0
52	CF_3	C(O)OEt	NMe_2	H	CF_3	0	0	0
53	CF_3	C(O)OEt	$NHC(O)CF_2CF_3$	H	CF_3	0	0	0
54	CF_3	C(O)OEt	$O(C_6H_4)NO_2$	H	CF_3	0	0	0
55	CF_3	C(O)OEt	$OC(O)(C_6H_4)NO_2$	Me	CF_3	0	0	0
56	CF_3	C(O)OEt	$S(C_6H_5)$	H	CF_3	0	0	0
57	CF_2CF_3	C(O)OEt	OH	H	OH	0	0	0
58	CF_3	C(O)OMe	OH	C(O)OMe	CF_3	0	0	0
59	CF_3	C(O)OMe	O(IPA)	C(O)OMe	CF_3	0	0	0
60	CF_3	C(O)OEt	$O(C_2H_4)NO_2$	H	CF_3	0	0	0
61	CF_3	C(O)OEt	Cl	H	CF_2Cl	0	0	0
62	CF_3	C(O)SMe	OH	H	CF_3	0	0	0
63	CF_3	C(O)SMe	O(IPA)	H	CF_3	0	0	0

[a] Evaluation of compounds was at a low, mid, and high rate of application represented by a range of chemical concentrations of 2.5–5.6, 8–11.2, and 13.5–16.8 kg/ha, respectively. Compounds were applied at approximately a 5-cm spike length stage of development. [b] Gametocide activity (100% sterility + partial sterility) is represented as a range of sterility observed under bagged spikes: 0 = 0–5%, 1 = 6–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100%. [c] IPA, diisopropylamine.

seeds were in a soft–hard dough stage of development, bagged spikes and unbagged spikes were evaluated for seed set. The number of seeds present on the spikes inside the glassine bags was used to quantify a compound's ability to produce infertility in wheat. Seed set within bagged spikes was used in classifying spike sterility: 1, 100% sterility, no seed set on the spike; 2,

Table II. Growth Chamber POE Evaluation of 4-Substituted Pyridinemonocarboxylates for Spike Sterility in Wheat at Three Application Rates



example	A	low[a]	mid	high
1	H	0[b]	0	0
2	OH	3	4	4
3	O(IPA)[c]	3	4	4
4	$OC(O)(C_6H_5)$	2	3	2
5	OC(O)Me	3	4	4
6	$OC(O)(C_6H_{11})$	3	4	4
7	$OC(O)CMe_3$	1	3	4
8	OC(O)Pr	3	4	4
9	$OC(O)CHMe_2$	–	4	4
10	OC(O)cPROPANE	3	4	4
11	$OC(O)N[CHMe_2]_2$	2	3	3
12	$OC(O)NMe(C_6H_5)$	4	4	4
13	$OC(O)NEt_2$	3	3	3
14	$OC(O)NPr_2$	2	1	3
15	$OC(O)N(C_6H_5)_2$	2	1	2
16	$OC(O)O(C_6H_5)$	4	4	4
17	$OC(O)(C_6F_5)$	3	4	4
18	$OS(O)_2(C_6H_4)Me$	3	4	4
19	$OC(O)(C_6H_4)CF_3$	3	4	4
20	OC(O)pyrrole	3	4	4
21	$OC(O)NMe_2$	2	3	3
22	$OC(S)NMe_2$	2	3	3
23	$OP(O)(OEt)_2$	3	4	4
24	$OP(S)(OEt)_2$	0	1	0
25	OC(O)SEt	3	3	3
26	SH	0	0	0
27	S(IPA)	0	0	0
28	Cl	0	0	0
29	OC(O)OMe	3	3	3
30	$OCH_2(C_6H_5)$	0	0	0
31	NH_2	0	0	0
32	NHMe	0	0	0
33	$NHC(O)CF_3$	0	3	2
34	$NHC(O)CF_2CF_3$	2	1	3
35	$NHC(O)CH_2Cl$	1	1	1
36	$NHC(O)CCl_3$	2	2	2
37	NHC(O)Me	1	0	1
38	$NHS(O)_2CF_3$	1	1	

[a] Evaluation of compounds was at a low, mid, and high rate of application represented by a range of chemical concentrations of 2.5–5.6, 8–11.2, and 13.5–16.8 kg/ha, respectively. Compounds were applied at approximately a 5-cm spike length stage of development. [b] Gametocide activity (100% sterility + partial sterility) is represented as a range of sterility observed under bagged spikes: 0 = 0–5%, 1 = 5–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100%. [c] IPA, diisopropylamine.

partial sterility, spikes containing one to five seeds (approximately 85% or greater sterility); 3, fertile spikes, spikes with more than five seeds. Spike sterility results for a respective compound presented in the tables represent the percentage of bagged spikes having 85% or greater sterility (percentage of spikes with 100% sterility plus percentage of spikes with partial sterility) and are presented as a single digit between zero and four representing a sterility range.

RESULTS AND DISCUSSION

Substitution changes on the pyridinemonocarboxylate backbone were evaluated with the best activity observed from 3-pyridinecarboxylic acid, 4-hydroxy-2,6-bis(trifluoromethyl) methyl ester (Table I, example 3), and its diisopropylamine salt (IPA) (Table I, example 5). Male sterility in the 75–100% range was consistently obtained with this molecule when sprayed at the 4–6-cm stage of spike development. This level of activity was observed in all growth chamber tests and at both the mid and high rates of application. Low application rates produced male sterility in the 50–75% range. With this structure (example 3) as the backbone, methodical chemical changes at a single position were synthesized and evaluated.

All of the conversions of the 3-methyl ester to larger esters, i.e., ethyl (Table I, examples 6 and 7), *n*-propyl (examples 8 and 9), isopropyl (examples 10 and 11), benzyl (examples 12 and 13), or thiomethyl ester (examples 61 and 62) as well as to 3-amides (examples 14 and 15) were inactive as CHAs. Hydrolysis of the 3-methyl ester to the free carboxylic acid (Table I, examples 16 and 17) resulted in compounds that were also inactive. Likewise, no activity was observed following decarboxylation at the 3-position (Table I, example 1).

Any changes in the 2- and/or 6-trifluoromethyl groups (keeping all other substituents on the pyridine ring constant) resulted in a total loss of activity. For example, the 2,6-bis(methyl) analogues (Table I, examples 22 and 23) as well as the 2,6-bis(difluoromethyl) (example 4) and 2,6-bis(chlorodifluoromethyl) (example 27) analogues were all inactive. Changes as small as the 6-CF_3 to a 6-CF_2H (example 26) or 6-CF_2Cl (example 25) also resulted in loss of activity as did more drastic changes as 6-CF_3 to 6-hydroxy (example 21) or 6-carbomethoxy (example 20).

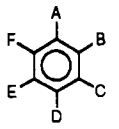
A number of 3-ethyl esters with a range of substitutions at the other positions demonstrated no chemical hybridizing activity. These results were expected since the conversion from the 3-methyl ester (Table I, example 3) to the 3-ethyl ester (example 6) of the lead compound resulted in the total loss of sterility.

Replacement of the hydrogen in the 5-position on the pyridine ring with either a methyl ester (Table I, examples 31 and 32), methoxy (examples 33 and 35), methyl (example 36), carboxyl (examples 37 and 38), or *tert*-butyl ester (example 40) resulted in a total loss of activity.

The only position of the pyridine backbone not to lose total chemical hybridizing activity following substitution was the 4-position. Various acyl, carbamyl, tosyl, and other derivatives capable of hydrolysis to the free hydroxyl (Table II, example 2) displayed a range of activity. As a result, all examples (Table II, examples 4–23, 25, 29) capable of hydrolyzing to the parent molecule showed various degrees of activity, probably related to the rate of hydrolysis to the free hydroxyl. However, none of the acyl derivatives were consistently superior in activity to the lead compound. When the 4-hydroxy was changed to a hydrogen (Table II, example 1), a chlorine (example 28), a mercapto (examples 26 and 27), a benzyloxy (example 30), an amino (example 31), or a methylamine (example 32), activity was lost.

When the 4-hydroxy was replaced by an amide functionality, only the ones containing electron-withdrawing groups [i.e., trifluoromethyls (Table II, example 33), pentafluoroethyl (example 34), and trichloromethyl (example 36)] exhibited activity. In general, spike sterility was observed when the substitution contained an acidic proton functionality. The proton on the substituted trifluoroacetamide (example 33), for example, is fairly acidic due to the electron-withdrawing effect of the fluorines and produced a level of male sterility approximately half that of the lead compound (Table II, example 2). Other amides were significantly less active. The 4-mercapto analogue (example 27) was probably too strong of a nucleophile and may react prior to reaching the target within the plant, thus resulting in no observed activity. The triflamide substitution (example 38) was not as active as expected even though the triflamide proton should be very acidic. The triflamide may be too acidic or the sulfonamide moiety may impart some negative effect, thus resulting in the

Table III. Growth Chamber POE Evaluation of Substituted Phenyl Analogues for Spike Sterility in Wheat at Three Application Rates



example	A	B	C	D	E	F	low[a]	mid	high
1	COOH	CF_3	H	CF_3	H	H	2[b]	4	4
2	COOH	CF_3	H	H	H	CF_3	0	2	2
3	COOH	H	CF_3	H	CF_3	H	0	0	0
4	COOH	CF_3	H	H	H	H	0	0	0
5	COO(IPA)[c]	CF_3	H	H	H	H	0	0	0
6	COO(IPA)	H	CF_3	H	H	H	0	0	0
7	COO(IPA)	H	H	CF_3	H	H	0	0	0
8	C(O)OME	CF_3	H	CF_3	H	H	0	0	0
9	COOH	H	H	OH	H	H	0		0
10	COOH	OH	OH	H	H	H	0		0
11	COOH	OH	OH	OH	H	H	0		0
12	COOH	H	H	Br	H	H	0		0
13	COOH	H	Br	OH	H	H	0		0
14	COOH	H	Br	OH	Br	H	0		0
15	COOH	H	Cl	H	Cl	H	0		0
16	COOH	Cl	H	Cl	H	H	0		0
17	COOH	H	Cl	Cl	H	H	0		0
18	COOH	Cl	Cl	H	Cl	H	0		0
19	COOH	H	Cl	OH	Cl	H	0		0
20	COOH	OMe	OMe	H	H	H	0		0
21	COOH	OMe	H	H	OMe	H	0		0
22	COOH	Me	Me	H	H	H	0		0
23	COOH	Me	H	H	Me	H	0		0
24	COOH	H	H	OH	H	H	0		0
25	COOH	OH	H	H	OH	H	0		0
26	COOH	H	OH	OH	H	H	0		0
27	OH	C(O)OMe	CF_3	H	CF_3	H	0	0	0
28	OH	CF_3	H	H	H	H	0	0	0
29	OH	H	CF_3	H	H	H	0	0	0
30	OH	H	H	CF_3	H	H	0	0	0
31	OH	NO_2	H	CF_3	H	H	0	0	0
32	O(IPA)	NO_2	H	CF_3	H	H	0	0	0
33	OH	H	CF_3	H	CF_3	H	0	0	0
34	OH	Cl	H	Cl	H	H	0		0
35	OH	Cl	H	Cl	Cl	H	0	0	0
36	CH_2OH	CF_3	H	H	H	H	0	0	0
37	CH_2OH	H	CF_3	H	H	H	0	0	0
38	CH_2OH	H	H	CF_3	H	H	0	0	0
39	CH_2OH	H	CF_3	H	CF_3	H	0	0	0
40	CH_2COOH	H	CF_3	H	H	H	0		0
41	C(O)Et	H	CF_3	H	CF_3	H	0	0	0
42	CN	H	CF_3	H	CF_3	H	0	0	0
43	CH_2CN	CF_3	H	H	H	H	0	0	0
44	NO_2	H	CF_3	H	CF_3	H	0	0	0
45	NH_2	H	CF_3	H	CF_3	H	0	0	0
46	C(O)Me	CF_3	H	H	H	H	0	0	0
47	C(O)Me	H	CF_3	H	H	H	0	0	0
48	C(O)Me	H	H	CF_3	H	H	0	0	0
49	C(O)Cl	CF_3	H	H	H	H	0	0	0
50	C(O)Cl	H	CF_3	H	H	H	0	0	0
51	C(O)Cl	H	H	CF_3	H	H	0	0	0
52	C(O)(C_6H_5)	CF_3	H	H	H	H	0	0	0
53	C(O)(C_6H_5)	H	CF_3	H	H	H	0	0	0
54	C(O)(C_6H_5)	H	H	CF_3	H	H	0	0	0
55	CH_2CN	CF_3	H	H	H	H	0	0	0
56	CH_2CN	H	CF_3	H	H	H	0	0	0
57	CH_2CN	H	H	CF_3	H	H	0	0	0
58	CHO	CF_3	H	H	H	H	0		0
59	CHO	H	CF_3	H	H	H	0		0
60	CHO	H	H	CF_3	H	H	0		0
61	CHO	H	OH	H	H	H	0		0
62	Cl	H	CF_3	H	H	H	0		0
				Pyridine Backbone					
63	-N-	H	COOH	COOH	H	H	0		0
64	-N-	COOH	H	H	COOH	H	0		0
65	-N-	H	COOH	H	COOH	H	0		0
66	-N-	COOH	COOH	H	H	H	0		0
67	-N-	COOH	H	COOH	H	H	0		0

[a] Evaluation of compounds was at a low, mid, and high rate of application represented by a range of chemical concentrations of 2.5–5.6, 8–11.2, and 13.5–16.8 kg/ha, respectively. Compounds were applied at approximately a 5-cm spike length stage of development. [b] Gametocide acitivity (100% sterility + partial sterility) is represented as a range of sterility observed under bagged spikes: 0 = 0–5%, 1 = 6–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100%. [c] IPA, diisopropylamine.

decreased activity. In general, groups in the 4-position (with all other positions on the pyridine ring constant) that could be hydrolyzed to a hydroxyl group or groups displaying an acidic proton in the 4-position produced spike sterility.

Placing the same substitution pattern of the lead pyridinemonocarboxylate compound (Table I, example 3) on a phenyl ring (Table III, example 27) resulted in a total loss of activity. These results indicated that, for sterility within the wheat spike, there was a very specific substitution requirement for the pyridinemonocarboxylates. The molecule requires a nitrogen in the ring, bis(trifluoromethyl)s in the 2,6-positions, a methyl ester in the 3-position, and a hydroxyl group (or a group that will hydrolyze to the hydroxyl) in the 4-position.

An exception to the above-stated observation is the case of 2,6-bis(trifluoromethyl)-3-pyridinecarboxylic acid (Table I, examples 18 and 19), which also exhibited good male sterility. This result led to the investigation of male sterility in the corresponding benzoic acid class of chemistry (Table III). As with the pyridinemonocarboxylates, spike sterility was very specific, with only 2 of 62 substituted benzoic acid and phenyl examples demonstrating any significant activity.

Excellent activity, similar to that of the lead pyridinemonocarboxylate, was observed with 2,4-bis(trifluoromethyl)benzoic acid at the mid and high rates of application. Interestingly, this substitution pattern of the benzoic acid is identical to its pyridine analogue (Table I, examples 18 and 19) which also displayed good CHA activity. The 2,6-bis(trifluoromethyl)benzoic acid (Table III, example 2) exhibited a lower level of activity while the 3,5-bis(trifluoromethyl)benzoic acid (Table III, example 3) was inactive. Benzoic acids containing only a single trifluoromethyl at either the 2- (examples 4 and 5), 3- (example 6), or 4- (example 7) position were inactive. Conversion of the free carboxylic acid of the most active benzoic acid (Table III, example 1) to a methyl ester (example 8) produced no activity. Benzoic acids containing various hydroxyl, bromo, chloro, methoxy, and methyl substitutions were all inactive. Ejiri et al. (1984) demonstrated inhibition of *Pinus* pollen tube elongation with 4-hydroxybenzoic acid. In our study, 4-hydroxybenzoic acid (Table III, example 24) was inactive in producing sterility in wheat.

Various commercially available hydroxyl, methyl ester, ethyl ester, nitro, amine, cyano, and chlorine substitutions in place of the carboxyl group were evaluated and found to be inactive for producing spike sterility. Many of these examples contain only a single substitution on the respective ring. Within the benzoic acid analogues, two trifluoromethyls are required for activity, which may explain the lack of activity observed with these other substituted phenyls in our study.

Within both areas of chemistry, specific molecules of substituted benzoic acids and pyridinemonocarboxylates demonstrated the potential of producing spike sterility in wheat. Very minor changes to the parent compounds result in complete loss of chemical hybridizing activity. Compounds from each of the two chemical areas were evaluated in the greenhouse and field to determine post-treatment cross-pollination required for hybrid seed production. Both areas of chemistry produced male sterility under these conditions while retaining female fertility as exhibited by the formation of hybrid seeds (data not presented). Chemical synthesis and additional biological data for the CHA activity of pyridinemonocarboxylates are described in the following patents: U.S. 4747871, U.S. 4609399, U.S. 4655816, U.S. 4936905, and EP 0276204.

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