*Scientific paper*

# The Effect of Fluorine Atom on the Synthesis and Composition of Gametocidal Ethyl Oxanilates

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***Dedicated to Prof. Dr. Boris Žemva on the occasion of receiving the Zois´ award for lifetime achievements.***

## Abstract

Three derivatives of ethyl oxanilate were synthesized in order to test their application as gametocides on the hermaphrodite plants like common wheat (*Triticum aestivum* L.). A substituent at para position (F, Br, CN) of aniline defined its reactivity towards diethyl oxalate **2**. Classical reaction in toluene was not selective and amidation occurred also at the second carbonyl groups of **2**. Alternative synthesis under solvent-free conditions with application of low pressure for removal of EtOH provided selectively with ethyl oxanilate **3a** and **3b**. 4-Cyanoaniline did not react selectively and the corresponding ethyl oxanilate **3c** was prepared from mono acid chloride of oxalic acid. Fluoro derivative **3a** was found to be the only one that gives stable aqueous suspension for its application as chemical hybridizing agent for common wheat, while bromo- **3b** and cyano- **3c** analogues were not soluble enough and suspension was stable for less than 2 hours. Fluoro derivative had shown the best induction of male sterility, while in comparison with standard chemical hybridizing agent they were substantially less toxic for plant.

**Keywords:** Chemical hybridizing agent; oxanilate, common wheat; heterosis; solvent-free synthesis

## 1. Introduction

For the exploitation of heterosis in hermaphrodite plant species (*i.e.* common wheat) induction of male sterility is a prerequisite for the out-crossing. In the past several approaches were proposed on the genetic level (e.g. cytoplasmic-genetic male sterility) and on the transgenic level (e.g. RNA interference, the expression of cytotoxic and cytostatic polypeptides) for the induction of male sterility in common wheat.[1] Unlike genetic and transgenic approaches for the production of hybrid seed, which are based on three distinct lines (♀ AA, isogenous line A'A', ♂ BB), in chemical induction of male sterility with chemical hybridizing agent (CHA) only parental components of a hybrid variety (a double-component system) are needed. In addition to this, the advantage of a chemical induction of male sterility is also the absence of complex genetic engineering and long-term input of male sterile cytoplasm using backcrossing.[2]

The connection between selective effect of a chemical on the metabolism of a plant in the sense of induction of male sterility (gametocidal activity) and production of a hybrid seed could be traced in as early as 1957. That year the method for the production of a hybrid cotton seed (*Gossipium hirsutum* L.) with the use of a chemical FW-450 (α,β-dichloro isobutyrate) was described.[3] Four basic groups of CHAs regarding the mode of action can be distinguished: growth regulators and substance suppressing the development of floral primordia, metabolism inhibitors, pollen germination inhibitors and microsporogenesis inhibitors.[4–7]

Only the group of microsporogenesis inhibitors were commercialized starting in the 1970s and 1980s with the development of a series of oxo-pyridazines (fenridazon – Hybrex®). In 1997, clofencet (2-(4-chlorophenyl)-

3-ethyl-2,5-dihydro-5-oxo-4-pyridazinecarboxylic acid – GENESIS®) was introduced, however it was forced to be withdrawn from circulation due to the ecological toxicity. Cinnoline derivatives have also gametocidal activity with SC-2053 1-(4-chlorophenyl)-1,4-dihydro-5-(2-methoxyethoxy)-4-oxocinnoline-3-carboxylic acid) being used as a commercial CHA CROISOR® 100.[5,7]

The desired CHA should have simple structure, which usually means also simple synthesis, very good selectivity between gametocidal activity and toxicity and should have lesser impact on the environment when used directly in the field. It is known that oxanilic acid is a valuable structural element based on natural building block – oxalic acid. Different bioactive oxanilates were developed and are functioning as anti-allergens and anti-asthmatics,[8,9] for lowering of lipid level in blood,[10] as caspase inhibitors for stroke treatment,[11] and as HIV-1 entry inhibitors.[12,13] It is also known that ethyl oxanilates inhibit the microsporogenesis and can be effectively applied as CHA in common wheat (*Triticum aestivum* L.).[14–16] A major drawback of ethyl oxanilates lies in difficult preparation of stable emulsions. For their field application, cyclohexanone is used as a solvent for emulsifiable concentrate together with Tween 80. Cyclohexanone functions only as a solvent without any additional value, and creates therefore an additional burden to the environment.

Fluorine atom is well known for its effect on bioavailability as well as altering the physico-chemical properties in relation to non-fluorinated compounds.[17] In our study, we report on the effect of the fluorine atom on the aromatic ring on the activity of ethyl oxanilates as CHAs in relation to bromo- and cyano- substituent as well as its effect on the formation of stable emulsions for field applications in terms of male sterility induction in common wheat (*Triticum aestivum* L.).

## 2. Experimental

Chemicals and solvents were purchased from commercial sources and used as received. Melting points were determined on a Büchi apparatus. NMR spectra were recorded on a Varian INOVA 300 spectrometer. Mass spectra were obtained on Q TOF Premier instrument with ESI method. FT-IR spectra were recorded on a Perkin Elmer Spectrum 400 spectrometer. IR analysis in real-time was performed on synthesis workstation EasyMax with ReactIR45 ATR-IR probe.

**Typical reaction procedure for amidation of 1a with $(COOEt)_2$ in toluene**. 1 mmol of 4-fluoroaniline **1a** (114 mg) was dissolved in 2 mL of toluene. Corresponding amount of diethyl oxalate **2** was added (1.05 or 5 equivalents) (0.1 mmol of boric acid was added where noted) and stirred under reflux for 5–8 hours. Reaction was followed by GC. Reaction mixture was diluted with ethyl acetate and washed twice with water. Organic fraction was dried with $Na_2SO_4$ and solvent removed under reduced pressure. The product was analyzed by NMR spectroscopy.

**Typical reaction procedure for solvent-free amidation of 1 with $(COOEt)_2$**. 1 mmol of aniline **1** was mixed with 5 mmol of diethyl oxalate **2** (0.1 mmol of boric acid was added where noted) and stirred at appropriate temperature. Reaction was followed by GC and analyzed by NMR spectroscopy.

**Synthesis of 3a**. 10.0 g of **1a** (90 mmol) and 65.7 g of **2** were mixed together and stirred at 100 °C for 12 hours. Reaction mixture was allowed to cool down to ambient temperature and product **3a** precipitated out of the reaction mixture. Hexane (50mL) was added and the product **3a** was filtered off with suction and washed with hexane. 16.7 g (88%) of white crystalline product was obtained:

***Ethyl 4-fluorooxanilate 3a***, mp 118–119 °C (mp 118–119 °C)[14]

$^1$H NMR (DMSO) 1.31 (t, J = 7 Hz, 3H), 4.31 (q, J = 7Hz, 2H), 7.20 (t, J = 9 Hz, 2H), 7.76 (dd, J = 9, 5 Hz, 2H), 10.85 (s, 1H).

$^{13}$C NMR (DMSO) 13.8, 62.4, 115.4 (d, 22Hz), 122.4 (d, 8Hz), 133.9, 155.4, 158.9 (d, 242Hz), 160.6.

IR ($cm^{-1}$) 3323, 2991, 2903, 1733, 1685, 1536, 1506, 1285, 1209, 1158, 1102, 1024.

Formation of diamide product **4a** was confirmed by comparison of NMR spectra of independently prepared sample (reaction of $(COCl)_2$ with two equiv. of **1a** in toluene provided pure **4a** as insoluble solid) and with known literature data.[18]

***$N^1$,$N^2$-bis(4-fluorophenyl)oxalamide* 4a**: $^1$H NMR (DMSO) 7.22 (t, J= 7 Hz, 4H), 7.87 (m, 4H), 10.91 (s, 2H).

**Synthesis of 3b**. 15.48 g of **1b** (90 mmol) and 65.7 g of **2** were mixed together and stirred at 100 °C for 27 hours. Reaction mixture was allowed to cool down to ambient temperature and product **3b** precipitated out of the reaction mixture. Hexane (50 mL) was added and the product **3b** was filtered off with suction and washed with hexane. 18.6 g (76%) of white crystalline product was obtained:

***Ethyl 4-bromooxanilate***, mp 159–160°C (mp 152–153 °C)[14]

$^1$H NMR (DMSO) 1.31 (t, J = 7 Hz, 3H), 4.30 (q, J = 7Hz, 2H), 7.53 (d, J = 9 Hz, 2H), 7.72 (d, J = 9 Hz, 2H), 10.89 (s, 1H).

$^{13}$C NMR (DMSO) 13.8, 62.5, 116.6, 122.4, 131.6, 136.9, 155.5, 160.4.

IR ($cm^{-1}$) 3336, 2990, 2990, 1698, 1589, 1540, 1477, 1398, 1256, 1068.

Formation of diamide product **4b** was confirmed by comparison of NMR spectra of independently prepared sample (reaction of $(COCl)_2$ with two equiv. of **1b** in toluene provided pure **4b** a insoluble solid) and with known literature data.[18]

***N*$^1$,*N*$^2$-*bis*(4-*bromophenyl*)*oxalamide* 4b**: $^1$H NMR (DMSO) 7.56 (d, J = 8 Hz, 4H), 7.82 (d, J = 8 Hz, 4H), 10.98 (s, 2H).

**Synthetic procedure for solvent-free amidation of 1a with $(COOEt)_2$ under reduced pressure**. 90 mmol of aniline derivative **1** was mixed with 5 mmol of diethyl oxalate (0.1 mmol of boric acid was added where noted) and stirred at appropriate temperature. Reaction was followed by GC and analyzed by NMR spectroscopy.

**Procedure for the synthesis of ethyl 4-cyanooxanilate 3c.** 99 mmol (11.08 g) of ethyl oxalyl chloride **5** was dissolved in 120 mL of ethyl acetate and cooled to 0 °C. 90 mmol (10.63 g) of 4-cyanoaniline **1c** was added slowly to the solution and left stirring for 1 h. Temperature was slowly raised to ambient temperature with stirring for additional 3 h. Reaction mixture was washed with 200 ml of saturated solution of $NaHCO_3$ and with 200 mL of water, dried with $Na_2SO_4$ and solvent removed under reduced pressure. Pure ethyl 4-cyanooxanilate **3c** (18.98 g, 97%) was obtained as white solid.

White crystaline product, mp 188–189 °C (mp 189 °C)[18]

$^1$H NMR (DMSO) 1.32 (t, J = 7 Hz, 3H), 4.32 (q, J = 7Hz, 2H), 7.83 (d, J = 9 Hz, 2H), 7.95 (d, J = 9 Hz, 2H), 11.17 (s, 1H).

$^{13}$C NMR (DMSO) 13.8, 62.7, 106.6, 118.8, 120.6, 133.2, 141.8, 155.9, 160.1.

IR ($cm^{-1}$) 3331, 2991, 2903, 2225, 1699, 1593, 1536, 1454, 1394, 1248, 1067.

**Preparation of different suspension concentrates of ethyl oxanilates 3 and stability of suspenisons.** Ethyl oxananilate **3** (1 g) was mixed with 2.2 g of emulsifier (Tween80 - polyoxyethylenesorbitan monooleate, Genapol® UD 050–$C_{11}$ oxo alcohol ethoxylate with 5 EO, Spartan™ – alkylamine ethoxylate propoxylate, SLES 70 – Sodium Lauryl Ether Sulphate) and appropriate amount of solvent (cyclohexanone or dimethylsulfoxide) were added to create a clear solution. This concentrate was slowly added to water under vigorous stirring to prepare the suspension.

**Preparation of spraying suspensions.** Ethyl oxananilate **3** (3 g) and 5g of Genapol® UD-50 and 24 mL of dimethylsulfoxide for **3a**, 120mL for **3b** and 135 mL for **3c** were mixed together. The suspension concentrate was stirred in 3L of water to prepare suspension for field application.

**The effectiveness of ethyl oxanilate 3 compared to standard CHA.** Three derivatives of ethyl oxanilate **3** and standard CHA (CROISOR® 100) were tested in the field experiment with 5 stripes 30 m × 1 m (the fifth stripe was control). Beside the active substance **3**, standard CHA (CROISOR® 100) and control (without treatment), we also included the subplot factor - phenophase and the sub-subplot factor – dose. Subplot factor phenophase represents three different lengths for the spike on the main stem (PP1: 5–10 mm, PP2: 10–15 mm, PP3: 15–20 mm) and the sub-subplot factor dose represent five different quantities of active substance (D1–700, D2–1400, D3–2100, D4–2800, D5–3500 g $ha^{-1}$). The subplot factor phenophase and the sub-subplot factor dose were presented in each stripe in the form of fifteen treatments with the surface of two square meters. Due to its broad commercial value and the already established use in the hybrid wheat seed production, the chemical hybridizing agent CROISOR® 100 was used in our study as standard CHA. Field experiments were repeated in two locations (central region: latitude 46°8'39.57''N, longitude 14°34'17.55''E; northeast region: latidude 46°37'22.58''N, longitude 16°7'44.10''E) and in two seasons (2009/10, 2010/11). French wheat variety Guarni (maintainer Florimond Desprez) that is known for its steady tillering and fast anther extrusion was used in the experiment. Seeds were sown by drilling in the first decade of October. The sowing density was 450 seeds/$m^2$, row to row distance was kept at 12.5 cm. Other optimum agronomic practices were also followed, which included adequate supply of mineral nitrogen in the form of 27 % calcium ammonium nitrate (EC 13/25, 70–90 kg/ha N; EC 31/32, 50–70 kg/ha N; EC 47/49, 70–90 kg/ha N), treatment with the sulphonylurea herbicide (EC 13/37), fungicide treatment (EC 29/37 – strobilurins; EC 51/69 – azols) and insecticide treatment (neonicotinoids). All derivatives **3** and standard CHA CROISOR® 100 were applied in the form of 0.1 % suspension at the appropriate phenophase (according to three mentioned spike lengths). Spray suspension was applied in the morning with ground application equipment using low-pressure and low drift flat fan nozzles (Lechler nozzle 110–04). Absorption potential of the active substance was estimated using the direct method measurements of the matrix water potential (tensiometer indicator – STEPS 40033) and green leaf area index measurements with Li-Cor LAI 2000 Plant Canopy Analyser.

**Measures of spikelet sterility, phytotoxicity and statistical analysis.** At heading ten spikes of each sub-subplot (2 $m^2$) and plot (8 × 12.5 cm × 750 cm) were isolated with a greaseproof paper bag to prevent any further cross-pollination. To study the spike sterility, the number of grains per spike on treated plants and the mean number of grains for thirty control spikes (untreated plants) were counted and the percent of spike sterility was computed using the following formula:

$$\% \text{ spike sterility} = (S_C - S_T) / S_C \times 100 \quad (1)$$

$S_T$: Number of grains per spike in bagged and treated plants.

$S_C$: Mean number of grains per spike in 30 bagged and untreated plants (control).

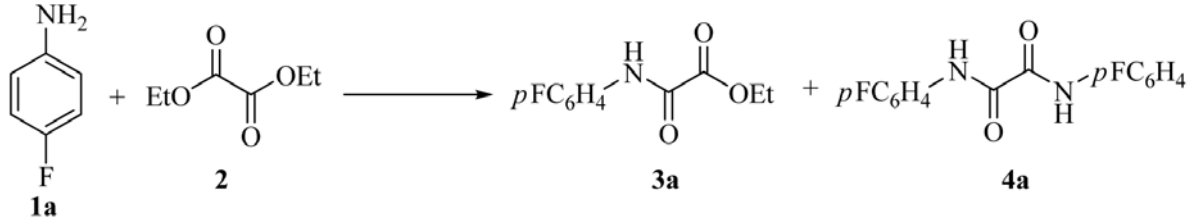
For the determination of phytotoxicity in the statistical analysis were also included the plant height and spikelet number. The mean values for thirty control plants were:

Season 2009/10
Northeast region: $S_C$ = 43.66; plant height 80.70; spikelet number 17.50
Central region: $S_C$ = 45.73; plant height 71.65; spikelet number 17.80
Season 2010/11
Northeast region: $S_C$ = 46.67; plant height 77.50; spikelet number 16.90
Central region: $S_C$ = 47.73; plant height 71.00; spikelet number 17.10

Statistical analysis was performed using the statistical package STATGRAPHICS Centurion XVI. The results from the field experiments were compared on the basis of significance at 5 and 1 % levels (p = 0.05 and 0.01).

## 3. Results and Discussion

**Synthesis of ethyl oxanilates 3.** The simplest synthesis of esters of oxanilic acid would be reaction of diester of oxalic acid with aniline derivatives (Scheme 1). This approach was reported to proceed in boiling toluene with 1.2 equivalents of diethyl oxalate and with azeotropic distillation of ethanol,[16] while boric acid as catalyst was also used.[14]




**Scheme 1.**

When we performed this reaction at a 1 mmol scale we obtained a mixture of targeted ethyl 4-fluorooxanilate (F-OxAn, **3a**) and a product of further reaction **4a** in a ratio 1:2 (Table 1, line 1). A catalytic reaction gave even higher proportion of **4a** (line 2). Our aim was to have very selective reaction that would led to simple isolation procedure. We tried to improve the yield of **3a** by applying higher amount of diethyl oxalate **2**. This strategy shifted the selectivity of the reaction towards the formation of **3a**, although catalytic reaction was again less selective (lines 3, 4). We explored further this reaction and as both starting compounds are liquid we performed the reaction in neat. With 1 equivalent of **2** very high reaction temperature had to be applied, while both products were formed (line 5). Again, boric acid shifted the reaction towards formation of higher amount of **4a**. When we increased the amount of **2** reaction in neat became completely selective with quantitative formation of **3a** in 28h. The reaction rate was increased by application of lower pressure in order to distil off the EtOH that is forming during the reaction (entry 8). This reaction setup provides with the selective formation of **3a** at higher reaction temperature. The formation of diamide **4a** was confirmed with the reaction of $(COCl)_2$ with 2 equivalents of **1a**, where **4a** was the only reaction product.

**Table 1.** The effect of reaction conditions on the synthesis of ethyl F-OxAn **3a**[a]

| Entry | 2 (equiv.) | Rect. cond. | 1a | 3a | 4a |
|---|---|---|---|---|---|
| 1 | 1.05 | $PhCH_3$, reflux, 8h | / | 33 | 67 |
| 2 | 1.05[b] | $PhCH_3$, reflux, 8h | / | 11 | 89 |
| 3 | 5 | $PhCH_3$, reflux, 5h | 20 | 80 | |
| 4 | 5[b] | $PhCH_3$, reflux, 5h | 66 | 17 | 17 |
| 5 | 1 | 160 °C, 2h | 45 | 33 | 22 |
| 6 | 1[b] | 160 °C, 2h | 23 | / | 77 |
| 7 | 5 | 100 °C, 12h | | 100 (88%)[c] | |
| 8 | 5[d] | 100 °C, 6h[d] | 15 | 85 | |
| 9 | 5[d] | 120 °C, 5h[d] | | 100 | |

[a] Product distribution was determined by $^1$H NMR spectra of isolated reaction mixture and is based on starting compound. [b]10 mol% of $B(OH)_3$ was added. [c]Value in parenthesis refers to the yield of the isolated product. [d]Reaction mixture was put under reduced pressure at 100 mmHg.

Above results show that solvent-free reaction occurs already at 100 °C and that azeotropic distillation can be replaced by reducing the pressure *i.e.* evaporation of EtOH at reduced pressure. In order to define which parameter activates the reaction we have followed the reaction at controlled reaction conditions in a synthesis workstation with ATR-IR in real time (Figure 1). We have mixed **1a** and **2** in a reactor equipped with a temperature sensor and an ATR-IR probe. Reaction temperature was first set to 100 °C and no reaction was observed. We have applied the reduced pressure to eliminate EtOH so that it was gradually reduced to 20 mmHg and reaction proceeded only slowly. However, as soon as we rose the temperature of the reaction to 120 °C the rate increased significantly and the reaction was finished within 20 minutes (Figure 2).

Similar reactivity was obtained with 4-bromoaniline **1b**, although it was even less reactive. Reaction in toluene did not proceed without elimination of EtOH from the reaction mixture or without the presence of $B(OH)_3$. Consequently, a higher proportion of diamide **4b** was formed. The reaction in neat proceeded slowly and contrary to reaction in $PhCH_3$, reaction was selective with exclusive formation of Br-OxAn **3b** (Scheme 2). 5 equivalents of **2** was sufficient for the synthesis of **3b**, while addition of

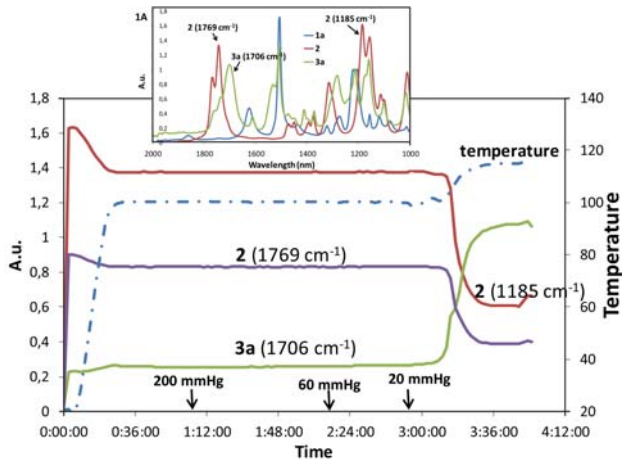
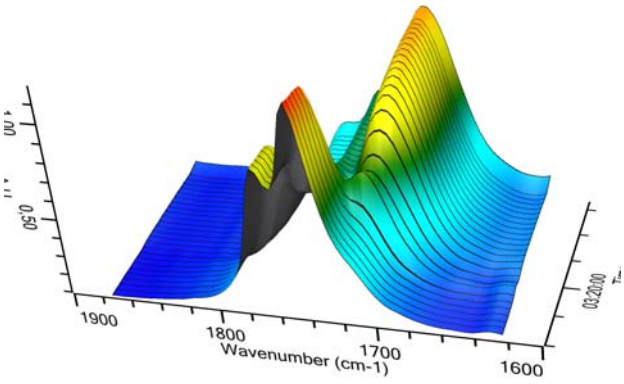



**Figure 1.** Real-time ATR-IR analysis of the amidation of diethyl oxalate **2** with 4-fluoroaniline **1a** under solvent-free conditions (inset 1A shows IR spectra of reaction components).



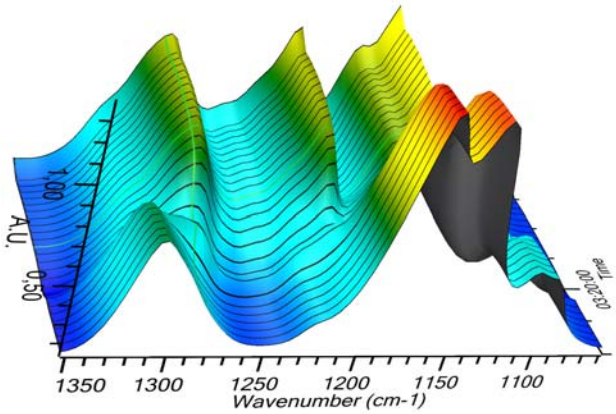



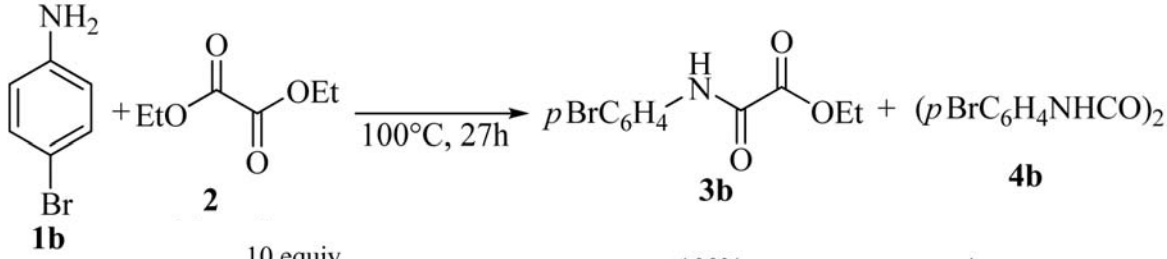

**Figure 2**. 3D IR spectra surface at the time of conversion of **1a** into **3a**

the catalyst again have a detrimental effect on the selectivity of amidation. Even smaller amount of **2** gave slow reaction with the formation of **4b** as a side product together with some unreacted **1b**.

4-Cyanoaniline **1c** is even less nucleophilic. Harsher reaction conditions had to be applied, nevertheless reaction at 160 °C was still very slow with only 61% conversion, while dimeric **4c** started to form already (Scheme 3). In a catalytic version of this reaction, temperature ought to be lowered to 100 °C in order to have selective conversion, although the conversion was still not complete. Therefore, we substituted diethyl oxalate with its mono acid chloride **5** and the reaction produced selectively to the formation of CN-OxAn **3c** with excellent yield.

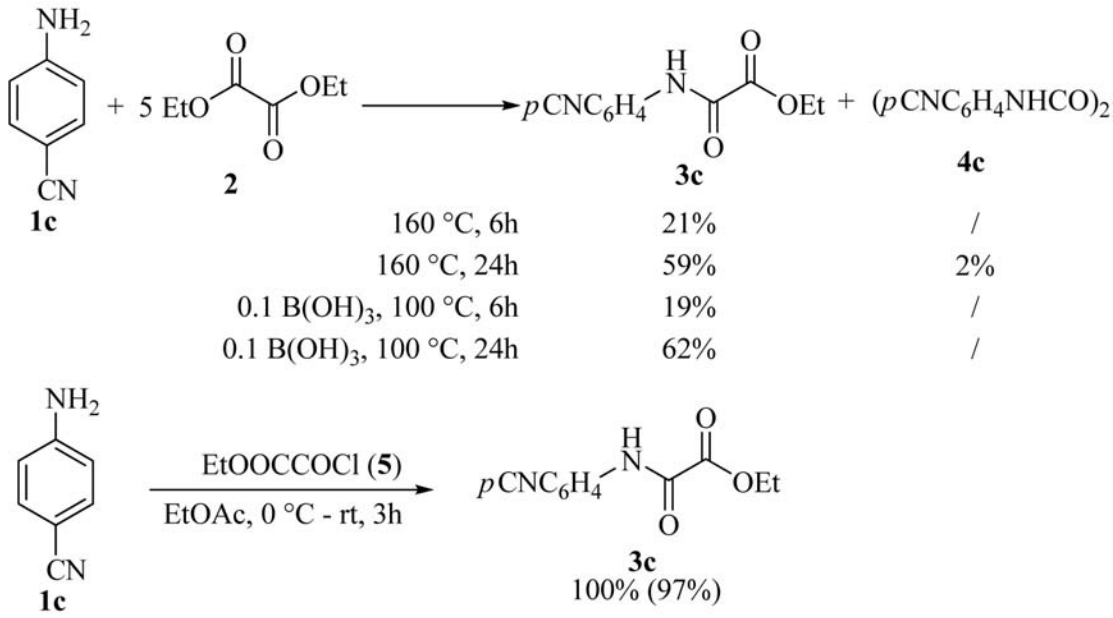
**Preparation of spraying suspension.** OxAn **3** were generally used as suspensions in water prepared from suspension concentrate of active compound in cyclohexanone with Tween80 as emulsifier. It turned out that it is very difficult to create a stable suspension due to low solubility and precipitation of ethyl oxanilates out of the water suspension. Therefore, fluorine atom can have an important effect due to its better solubility.

First, we have determined the solubility of all three compounds **3** in cyclohexanone (CyHex), acetonitril (MeCN) and dimethylsulfoxide (DMSO) by slowly adding solvent to **3** and stirring until clear solution was obtained. As could be seen from the Table 2 the effect of fluorine on the aromatic ring clearly increases the solubility. In relation to CN-OxAn **3c**, **3a** is of order of magnitude more soluble, which indicates that fluorine derivative




| | | |
|---|---|---|
| 10 equiv. | 100% | / |
| 10 equiv., 10 mol% $B(OH)_3$ | 100% | / |
| 5 equiv. | 100% (76%) | / |
| 5 equiv., 10 mol% $B(OH)_3$ | 62% | 38% |
| 2 equiv. | 70% | 7% |

**Scheme 2.**




**Scheme 3.**

**3a** will be easier to use in agrochemical spraying compositions.

**Table 2**. Solubility (g/L) of ethyl oxanilates **3**

| | cyHex | MeCN | DMSO |
|---|---|---|---|
| F-OxAn **3a** | 154 | 129 | 151 |
| Br-OxAn **3b** | 48 | 19 | 27 |
| CN-OxAn **3c** | 14 | 12 | 17 |

In the next step we studied, how a substituent affects the stability of suspensions formed upon mixing with water. It is evident that addition of emulsifier is necessary not only to stabilize the emulsion or suspension but also to increase the solubility of **3** in organic solvent. Otherwise, the amount of solvent would be too high for field experiments (*i.e.* cca 80 L per ha for **3c**). We have tested different emulsifiers in order to test the stability of suspensions (Table 3). The amount of emulsifier was set to correspond to approximately 0.2% of final spraying suspension. The amount of solvent was regulated so that clear solution is formed. Regardless on the emulsifier used, fluorine derivative **3a** formed stable solutions in all examples. Even after 24h was suspension bright and no precipitate formed. Different case was observed with **3b** and **3c**, where suspensions were stable only for 15 minutes to two hours, after that solid started to precipitate at the bottom. Consequently, suspension is no longer suitable for application. On the other hand, suspension with fluorine derivative **3a** is stable and therefore, the application is much more practical and could be applied during prolonged reaction time.

**Table 3**. Composition of suspension concentrate

| | | Emulsifier[a] | | | |
|---|---|---|---|---|---|
| | **Solvent** | **Tween80** | **UD-50** | **Spartan** | **SLES** |
| F-OxAn | CyHex | 1/2.5/7 | 1/2.3/7 | 1/2.2/7 | 1/2.3/7 |
| **3a** | DMSO | 1/2.2/14 | 1/2.3/8 | 1/2.3/7 | 1/2.1/7 |
| Br-OxAn | CyHex | 1/1.6/10 | 1/2.1/22 | 1/2.2/22 | 1/2.1/22 |
| **3b** | DMSO | 1/2.9/29 | 1/2.6/39 | 1/2.1/39 | 1/2.1/39 |
| CN-OxAn | CyHex | 1/3.9/22 | 1/2.1/70 | 1/2.1/76 | 1/2.1/76 |
| **3c** | DMSO | 1/2.1/24 | 1/2.1/44 | 1/2.1/63 | 1/2.1/63 |

[a] **3** (g) /emulsifier (g) /solvent (mL).

**Performance evaluation of modified derivatives of oxanilates.** Among oxanilic acid derivatives, which were investigated in our study, according to its efficiency in terms of male sterility induction, the F-OxAn **3a** stood out, followed by Br-OxAn **3b** and CN-OxAn **3c** (F > Br > CN) (Table 4). A preliminary study of Chakraborty and Devakumar showed that ethyl esters of oxanilic acid, such as ethyl 4-fluoro-oxanilate **3a** achieve a high percentage of male sterility in common wheat (> 98 %) in the phenophase, where the spike on the main stem has a length of 6–9 mm.[19] Our study also revealed that ethyl 4-fluoro-oxanilate **3a** induces a high percentage of male sterility (> 98 %), but in the phenophase where the spike on the main stem has a length of more than 9 mm (Figure 3). Therefore, fluorine derivative **3a** show much stronger effect on the sterility, while it does not have significant toxic effect on the plant as shown by plant height. Although CN-OxAn **3c** has smaller gametocidal activity and also toxicity, all three derivatives had very similar effect on the spikelet formation and thus on the ability to produce hybrid seed.

**Table 4**. The effect of substituent on the phenyl ring of ethyl oxanilate **3** on male sterility, plant height and spikelet number in common wheat (*Triticum aestivu*m L.)[a]

| Active substance | Male sterility [%] | Plant height [cm] | Spikelet number [n] |
|---|---|---|---|
| F-OxAn **3a** | 75,0 ± 0,4 | 61,7 ± 0,2 | 17,78 ± 0,08 |
| Br-OxAn **3b** | 71,7 ± 0,4 | 60,1 ± 0,2 | 17,80 ± 0,08 |
| Cn-OxAn **3c** | 42,0 ± 0,4 | 66,3 ± 0,2 | 17,76 ± 0,08 |
| **Standard CHA** | 96,5 ± 0,4 | 44,3 ± 0,2 | 15,45 ± 0,08[b] |

[a] Average values for three ethyl oxanilates and standard CHA (average values are calculated according to experimental procedure: two locations, two seasons, five different doses and three phenophases).

More detailed comparison was made with fluorine derivative **3a** and the standard CHA (**Figure 3**). Phytotoxic activity is represented by the plant height and it could be seen that the standard CHA show toxic effect for the treated plants already at the recommended parameters (phenophase with spike length on the main stem from 15 to 20 mm (PP3), the recommended dose (1400 g $ha^{-1}$) and recommended application conditions (min. air temperature 12 °C, min. relative air humidity 50 %) – the reduction of plant height was more than 30 %. In comparison of the derivative of oxanilic acid that significantly achieve the highest percentage of male sterility (**3a**), with the standard CHA, related to the the third dose (2100 g $ha^{-1}$) there was no statistically significant difference in the percentage of male sterility, but from data about plant height the high toxic impact of the CROISOR® 100 on treated plants is evident (**Figure 3**). In the dose recommended for standard CHA (1400 g active substance per hectare), the efficacy of the derivative **3a** grew with the development of floral primordia, while there were no significant toxic effect even at larger doses.

# 4. Conclusion

The effect of substituent on the phenyl ring in ethyl oxanilates **3**, that are known for its gametocidal effectiveness (inhibitors of microsporogenesis), was studied with particular interest in the effect of fluorine atom on the synthesis and bioactivity. In the synthesis of **3** the most reactive aniline was 4-fluoroaniline **1a**, while the other two tested ones (bromo **1b** and cyano **1c** derivatives) were less nucleophilic. The substituent in **3** also importantly affects the ability to form stable suspensions in water so that they can be applied on the field. Fluorine was again the substituent of choice as it was the only one that formed stable suspension. In parallel, bioactivity of F-OxAn was also the highest amongst the three studied gametocidal compounds.

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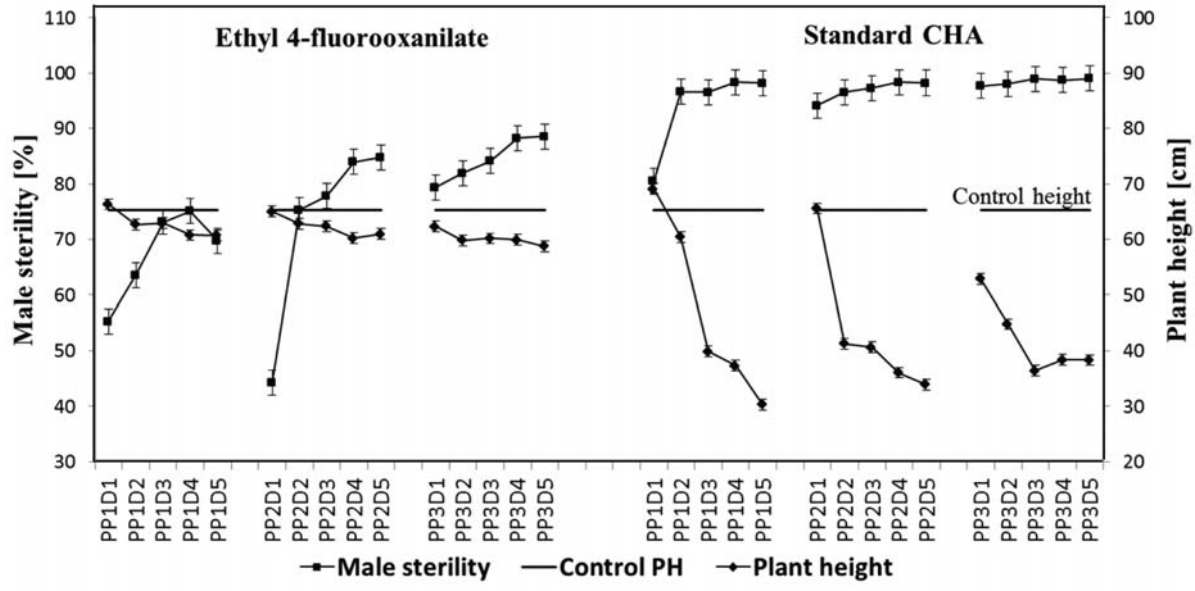


**Figure 3**. The impact of phenophase of application and the dose of active compound (**3a**, standard CHA) on induction of male sterility and plant height.

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## Povzetek

Sintetizirali smo tri derivate etil oksanilata z namenom testiranja njihove učinkovitosti kot gametocidov za hermafroditne rastline, kot je navadna pšenica (*Triticum aestivum* L.). Substituenta na para mestu anilina (F, Br, CN) vpliva na reaktivnost le-tega za reakcijo z dietil oksalatom **2**. Reakcija pod klasičnimi pogoji v toluene ni selektivna in amidiranje poteče tudi na drugi esterski skupine **2**. Alternativna sinteza v reakcijskih pogojih brez topila in z uporabo znižanega tlaka za odstranjevanje EtOH se je izkazala za učinkovito, saj sta selektivno nastala etil oksanilata **3a** and **3b**. 4-Cianoanilin tudi v teh pogojih ni reagiral selektivno in smo odgovarjajoči etil oksanilat **3c** pripravili iz kislinskega klorida EtOCOCOCl. Najbolj stabilne vodne suspenzije za nadaljnjo aplikacijo za kemično hibridizacijo pšenice je tvoril fluorov derivat **3a**, medtem ko sta bila bromo- **3b** in ciano- **3c** analoga manj topna, njune suspenzije pa stabilne manj kot 2 uri. Največjo učinkovitost za indukcijo moške sterilnosti pri pšenici je imel fluorov derivat **3a**, v primerjavi s standardno učinkovino za kemično hibridizacijo pa je izkazal občutno manjšo toksičnost za rastlino.