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# Impact of Pesticide Applications in Cotton Agroecosystem and Soil Bioactivity Studies II: Nitrification Dynamics

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**Abstract**: Field experiments were conducted to study the effect of pesticide applications on soil nitrification dynamics in cotton agroecosystem. The pesticides either alone or in combination (mixture) were applied according to the normal agricultural application rates in test, farmer (with pesticide applications) and control (without pesticide applications) fields. Soil samples were collected before and after two days, following pesticide applications and at sowing, before pesticide application, after all pesticide applications, at harvest and at post harvest time during the crop seasons 1997-1999. Soils were incubated under laboratory conditions using ammonium sulfate as substrate. In 1997 crop season soil samples were incubated for 28 days with a substrate of 144 $mgkg^{-1}$ soil while in 1998-1999 the incubation period was 14 days with substrate of 212 $mgkg^{-1}$ soil. The soil samples were analyzed for total ($NO_2$ + $NO_3$) N content using steam-distillation method. Othofonprox, profenophos + cypermethrin and bifenthrin + endosulfan inhibited while, endosulfan, imidachloprid, methamidophos, endosulfan alongwith dimethoate, profenophos + alphmethrin, chlorpyrifos + tralomethrin + acetamiprid and cyhalothrin + profenophos + diafenthiuron stimulated the nitrification. All other pesticidal applications have no effect on this parameter. Samples collected at different intervals of time from all the fields in three years study showed no differences in nitrification from sowing to harvest. The variations observed, in general, being very week and transient and resulting in a recovery of nitrification.

**Key words:** Pesticides, soil, nitrification, risk assessment and long-term monitoring

## Introduction

Biotic processes mainly regulate nitrogen cycling and microorganisms play a crucial role in controlling the fate of N-fertilizer in soil. Nitrification, the biological formation of nitrite or nitrate from compounds containing reduced nitrogen, is mainly carried out by *Nitrosomonas* and *Nitrosobacter* sp. The importance of nitrifying microorganisms rests upon their capacity to produce nitrate, which is the major nitrogen source assimilated by higher plants. The effect of pesticides on nitrification is determined by measurement of the changes in the level of nitrite and nitrate-nitrogen in the soil. Application of pesticides and their residues are known to have an impact on microbial population (Ambrogioni *et al.*, 1987) in soil which may lead to stimulation, decrease or modification of soil biological processes such as nitrification (Andrea *et al.*, 1999), ammonification (Bardiya and Gaur, 1968) and other processes which are essential for soil health, fertility status and crop production.

Small and medium rates of Nuvacron (monocrotophos) and Superacide (methidathion) are safe for insect pest control without any danger to the nitrification process in soil, however, use of higher rates of both the insecticides can be depressive on the nitrification in soil (Bollen and Tu,1971). The recommended rates of the insecticides DDT, aldrin, endrin and diazinon did not suppress the nitrifiers at all. Insecticides at manifold higher doses than the recommended ones, however, exerted deleterious effect on nitrification (Bremner and Keeney, 1965). Many investigations have been carried out on the effect of single application of insecticides on microbes and their functions and mostly these investigations are under laboratory conditions. In general, observed effect were minor and short-lived (Chandra, 1987), however, not much is known about the more realistic situation, where different insecticides are used together. These might arise additional stress from use of mixtures and sequences of pesticides because of accumulation of toxic agents or the formation of new compounds which may be more toxic than the original ones. Keeping in view, the heavy repeated long-term applications of pesticides in cotton agroecosystem, the present study was undertaken to investigate the impact of various sequentially applied pesticides at recommended doses as per normal practices on nitrification dynamics.

## Materials and Methods

**Insecticides**: The insecticides were purchased locally for the use of cotton plant protection. The insecticides were used individually or in combinations (mixtures). They were applied in the plots at the recommended rates of field application. Their general names and dose of application are given below:

**1st crop season**: Endosulfan + Dimethoate, Profenophos + Cypermethrin, Profenophos, Endosulfan, Carbosulfan + Fenvalerate, and Fenpropathrin were applied @ 1000+500, 600, 1000, 1000, 600+300 and 330 ml acre $^{-1}$ respectively

**2nd crop season**: Methamidophos, Monocrotophos, Profenophos + Diafonthiuson, Profenophos + Alphamethrin, Bifenthrin + Endosulfan, Bifenthrin + Acetamiprid, Profenophos + Ethion, Bifenthrin + Ethion, Propargite, Bifenthrin + Carbosulfan + Chlorpyriphos were applied @ 500, 1000, 1000+250, 800+330, 250+1000, 250+125(g), 1000+600, 250+1500, 330, 250+500+1000 ml $acre^{-1}$ respectively.

**3rd crop season** : Methamideophos, Othofonprox, Endosulfan, Endosulfan+Methamideophos, Imidachloprid, Profenophos+ Cypermethrin, Chlorpyriphos+ Trolomethrin+ Acetamiprid, Cyhalothrin + Profenophos, Diafonthiuron, Profenophos + Trolomethrin, Carbosulfan and Imidachloprid were applied @ 500, 125, 1000, 1000+500, 250, 600+200, 1000+100+125+, 330+1000, 1000+120, 500+250 ml $acre^{-1}$ respectively.

**Field site and Insecticide spray schedule**: The field site was selected at NIAB experimental farm, Faisalabad. The following three duplicate plots, size 500 $m^2$, were made to layout the experiment: a) Control soil without any pesticide treatment + fertilizers + cotton plants and treated soils i.e. b) test soil with pesticide treatment + fertilizers + cotton plant, c) treated soil from actual farm cotton plot (Farmer soil). Cotton varieties NIAB-78 and CM-240 were sown @ 20 kg $ha^{-1}$ during 1997-99 crop seasons. Seeds were first delinted using commercial $H_2SO_4$ and washed thoroughly with tap water. Fertilizers DAP and Potash @ 50 kg $acre^{-1}$ were applied at the time of sowing and Urea @ 50 kg $acre^{-1}$ at the time of boll formation. The soil used was clay loam with inorganic fraction, contained 22.79% clay, 26.36% silt, 50.58% sand and 23.03% clay, 23.12% silt, 53.86% sand for control and treated respectively. The organic matter of upper 15cm layer was 0.48 and 0.79%; saturation, 32.33 and 35.67 %; pH (saturated paste), 7.86 and 7.72; ECe, 0.39 and 0.21 $mScm^{-1}$ ; Total nitrogen, 0.05 each for control and treated fields.

**Soil Sampling**: Soil samples (0-15 cm) were taken at random by soil auger from 12 – 15 cores (2.5cm diameter) from each field and mixed thoroughly to prepare composite sample. Plant material and other debris were removed from the sample by hand and the soil was sieved using 2mm mesh. The soil samples were collected only at sowing time, before and after each pesticide application at harvest and post harvest during the crop seasons 1997-99. Samples were brought to the laboratory and stored at 4 $^{O}C$ till analyses were conducted. Temperature, relative humidity and rainfall were recorded at sampling time throughout the experimental period.

**Incubation experiments**: 5.0 g treated soil (oven dry basis) was taken in a plastic bottle and ammonium sulfate as a substrate solution (144 mg N $kg^{-1}$ in 1st year and 212 mg N $kg^{-1}$ in 2nd and 3rd years) was added to the soil. Plastic bottle was closed with 1.5mm thick stopper and incubated at 30 $^{O}C$ for 4 and 2 weeks in 1st, 2nd and 3rd years respectively. Moisture was maintained during the incubation at 60 % of the soil moisture holding capacity. A control sample was also run simultaneously without ammonium sulfate (IAEA, 1995 and Kandeler, 1996)

**Determination of $(NO_2+NO_3)$-N:** Nitrite + Nitrate was determined by steam distillation method from soil sample to produce ammonia ($NH_3$), using heavy MgO and Devarda's alloy (Dijik,1987). The distillate was collected in saturated $H_3BO_3$ and titrated to pH 5 with dilute $H_2SO_4$.

## Results and Disscusion

In the nitrogen cycle of the soil, nitrate has a central position since it is the most bioavailable nitrogen for plants. Nitrification is a test suitable for assessing the side effect of pesticides on soil non-target organisms because of its sensitivity and the agronomic significance of this process (Dijk, 1987). Soil microbial parameter "nitrification" of cotton agroecosystem was monitored from 1997 to 1999, during the three growing seasons, in order to assess the effects of repeated long-term pesticides usage in cotton field soils. In 1997 crop season, endosulfan along with dimethoate increased while profenophos + cypermethrin inhibited the nitrification after their application. All other pesticides have no effect on nitrite and nitrate contents in comparison with control soil (Table 1 and 4). Because many of the insects, developed resistance to some chemicals especially on cotton crop so newer chemicals (pesticides) are being substituted whenever possible. It is important to know that if these alternative pesticides have any pronounced influence on soil nitrification. In 1998-99 crop seasons some new pesticides alone or in combination with other, were used in order to protect cotton crop. Bifenthrin + endosulfan significantly inhibited the nitrification while prophenofos along with alphamethrin stimulated it after 2 days of their application in farmer field (Table 2). No such significant effect of these pesticides was noticed as compared to control (Table 5), because all the three fields; test, farmer and control have same nitrite + nitrate contents except methamidophos which stimulated the nitrification in farmer field as compared to control field during this season. In 1999, othofonprox decreased, while endosulfan, imidachloprid and a mixture of chlorpyrifos + trolomethrin + acetamiprid and cyhalothrin + profenophos + diafenthiuron stimulated the nitrification after their applications. All other pesticides have no effect on soil nitrification. Pesticide applications indicated that mixed results were observed for the effect of pesticides on soil nitrification. Endosulfon showed different trend of nitrification during crop seasons due to the variability of soil texture. Similar results were reported by Andrea *et al.* (1999), that nitrate was stimulated by the application of enodsulfan in Tatue soil but in Sao Paulo it was inhibited. Chandra (1987) found that dieldrin and heptachlor suppressed the nitrification in heavy clay, a sandy loam and 2 loam soils upto 8 weeks; at 16 weeks nitrification increased and toxic effects disappeared in the heavy clay and a mountain loam soil. Bardiya and Gour (1968) found that inhibition of nitrification of $(NH_4)_2SO_4$ in sandy loam by 25 $\mu g\ g^{-1}$ or higher concentrations of insecticides was temporary and followed the order of lindane (3 weeks inhibition)> dieldrin (2 weeks)> aldrin (1 week). Bollen and Tu (1971) found no significant effect on nitrification from eldrin treatments at 1 and 10 $\mu g\ g^{-1}$ in soils to which $(NH_4)_2SO_4$ was added. Tarrant *et al* (1972) found that the low concentration of DDT (aerial application) in the soil did not show any effect on nitrification rate and amount of $NO_3$-nitrogen. From the majority of the reports it would appear that the organochlorine insecticides are moderately toxic to the nitrifying bacteria. Propanil, a herbicide was sufficiently inhibitory to autotrophic nitrifiers in the soil to cause serious depression of nitrification at field rates of application (Debona and Audus, 1968). Several insecticides, for example, aldrin, lindane and parathion were tested by Garretson and San Clemente (1968) against pure culture of either *N eurapaea* or *Nitrobacter agillis* and found inhibitory effect at 1 to 10 $\mu g\ g^{-1}$ , but even these concentrations are more than would normally occur in the soil.

At normal field rate of pesticide application, organophosphates and pyrethroids stimulated the nitrification with other pesticides like acetamiprid and diafenthiuron in mixture form. Naumann (1970b) reported that malathion at 1000 $\mu g\ g^{-1}$ delayed nitrification by N. agillis while at 10 $\mu g\ g^{-1}$ did not delay the nitrification activity of *N. europaea*. Methyl-parathion, highly toxic but short-lived insecticide is rather rapidly decomposed by microorganisms in soil and stimulates the increase of various physiological groups including nitrifiers. Sahrawat *et al.* (1987) also reported inhibition of nitrification by parathion and malathion insecticides. A marked suppression of ammonium oxidizers by chlorophyrifos was observed by Sivasithamparam , 1969, 3 weeks after treatment but after 3 month there was a sharp increase in their numbers. Similar

Table 1: Impact of pesticide application on nitrification in farmer field, 1997

| Pesticides | BPA* | APA** | Mean |
|---|---|---|---|
| | (µg ($NO_2$+$NO_3$)-N $g^{-1}$ dry soil | | |
| Endosulfan + dimethoate | 11.86 d*** | 34.09 a | 27.95 A |
| Profenophos + cypermethrin | 21.81 bcd | 28.72 ab | 20.29 ABC |
| Profenophos + cypermethrin | 28.02 ab | 11.68 d | 20.20 ABC |
| Profenophos | 19.26 bcd | 21.75 bcd | 20.51 AB |
| Profenophos + cypermethrin | 24.21 abc | 13.36 cd | 18.79 BC |
| Endosulfan | 13.06 cd | 10.65 d | 12.01 C |
| Carbosulfan + fenvalerate | 21.94 bcd | 18.77 bcd | 20.36 ABC |
| Fenpropathrin | 11.51 d | 12.53 cd | 12.02 C |
| Mean | 19.08 A | 18.95 A | |

*Before pesticide applications ** After pesticide applications ***Means followed by same letters are statistically non-significant. LSD (5 %): For treatments; ns, for interaction (P x T); 10.78. LSD (1%): For pesticides; 7.62, for interaction (P x T); 10.78.

Table 2: Impact of pesticide applications on nitrification in farmer field, 1998

| Pesticide | BPA | APA | Mean |
|---|---|---|---|
| | (µg ($NO_2$+$NO_3$)-N $g^{-1}$ dry soil | | |
| Methamidophos | 104.17 ef | 101.54 ef | 102.85 F |
| Monocrotophos | 108.51 f | 74.18 f | 91.37 F |
| Profenophos + diafenthiuron | 159.42 bcd | 186.87 b | 173.14 BC |
| Profenophos + alphamethrin | 163.67 bcd | 221.71 a | 192.69 AB |
| Profenophos + endosulfan | 218.36 a | 182.17 b | 200.27 A |
| Profenophos + ethion | 158.20 bcd | 168.29 bcd | 163.24 CDE |
| Bifenthrin + ethion | 142.94 d | 145.37 cd | 144.15 E |
| Propergite | 144.43 d | 157.62 bcd | 151.03 DE |
| Bifenthrin + carbosulfan + chlorpyrifos | 178.02 bc | 160.12 bcd | 169.07 CD |
| Mean | 152.85 A | 155.30 A | |

LSD (5 %): For pesticides; 20.58, for treatments; ns, for interaction (P x T); 29.11. For other explanation see Table 1.

Table 3: Impact of pesticide applications on nitrification in farmer field, 1999

| Pesticide | BPA | APA | Mean |
|---|---|---|---|
| | (µg ($NO_2$+$NO_3$)-N $g^{-1}$ dry soil | | |
| Methamidophos | 184.50 cdef | 196.68 bcd | 190.59 CDE |
| Othofonprox | 192.74 bcde | 147.91 h | 170.32 F |
| Endosulfan | 147.91 h | 190.12 bcde | 169.02 F |
| Endosulfan + Methmidophos | 185.96 cdef | 176.98 efg | 181.47 DEF |
| Imidachloprid | 185.51 cdef | 210.22 abc | 197.87 BCD |
| Profenophos+(Profenophos+cypermethrin) | 201.11 bcde | 184.38 cdef | 193.22 BCDE |
| Chlorpyrfos + trolomethrin + acetamiprid | 162.69 fgh | 193.32 bcde | 178.01 EF |
| Cyhalothrin + profenophos | 155.71 gh | 204.73 bcd | 180.22 EF |
| Diafenthiuron | 208.65 abcd | 206.22 abcd | 207.44 ABC |
| Profenophos + trolomethrin | 213.86 ab | 203.71 bcd | 208.73 AB |
| carbosulfan | 200.84 bcde | 183.03 def | 191.84 BCDE |
| Imidachloprid | 220.78 a | 212.90 ab | 216.84 A |
| Mean | 189.27A | 192.51A | |

LSD (5 %): For pesticides; 7.62, for treatments; ns, for interaction (P x T); 10.78. LSD (1%): For pesticides; 7.62, for treatments; ns, for interaction (P x T); 10.78.

Table 4: Impact of pesticide applications on nitrification in untreated and treated fields, 1997

| Pesticides | Control field | Test field | Farmer field | Mean |
|---|---|---|---|---|
| | (µg ($NO_2$+$NO_3$)-N $g^{-1}$ dry soil | | | |
| Endosulfan+dimethoate | 7.40 ghi | 10.74 fghi | 34.09 a | 17.40 ABC |
| Profenophos + cypermethrin | 24.61 abcd | 12.12 efghi | 27.72 ab | 21.84 A |
| Profenophos + cypermethrin | 10.87 fghi | 4.54 I | 11.68 efghi | 9.03 D |
| Profenophos | 11.05 fghi | 6.34 hi | 21.76 bcde | 13.05 CD |
| Profenophos + cypermethrin | 17.60 cdefg | 12.18 efghi | 13.36 efghi | 14.38 BCD |
| Endosulfan | 13.61 efghi | 11.99 efghi | 10.66 fghi | 12.09 CD |
| Carbosulfan + fenvalerate | 7.60 ghi | 13.74 efghi | 15.82 defgh | 12.39 CD |
| Fenpropathrin | 18.90 cdef | 25.78 ab | 12.53 efghi | 19.07 AB |
| Mean | 13.95 B | 12.18 B | 18.57 A | |

LSD (5%): For pesticides (P) 5.06; for fields (F) 3.10; for interaction (P x F) 8.77. LSD (1%): for pesticides (P) 6.87; for fields (F)4.21;for interaction (P x F) 11.90.For other explanation see Table 1

Table 5: Impact of pesticide applications on nitrification ($\mu$g $(NO_2+NO_3)$-Ng$^{-1}$ ) in untreated and treated fields, 1998

| Pesticides | Control field | Test field | Farmer filed | Mean |
|---|---|---|---|---|
| | ($\mu$g $(NO_2+NO_3)$-N g$^{-1}$ dry soil | | | |
| Methamidophos | 73.22 gh | 46.98 h | 101.54 fg | 73.91 E |
| Monocrotophos | 79.01 gh | 76.09 gh | 74.18 gh | 78.57 E |
| Profenophos + diafenthiuron | 188.41 ab | 174.14 bcd | 186.87 abc | 183.14 AB |
| Profenophos + endosulfan | 185.80 abc | 213.84 a | 182.18 abc | 193.95 A |
| Profenophos + ethion | 160.71 bcd | 172.58 bcd | 168.30 bcd | 167.19 BC |
| Bifenthrin + ethion | 150.40 cd | 155.13 bcd | 145.37 de | 150.30 CD |
| Propergite | 116.27 ef | 158.05 bcd | 157.62 bcd | 143.98 CD |
| Bifenthrin+carbosulfan+chlorpyrifos | 169.20 bcd | 186.26 bcd | 160.12 bcd | 165.38 BC |
| Mean | 140.43 A | 145.38 A | 147.02 A | |

LSD (5%): For pesticides (P) 18.24; for fields (F) ns; for interaction (P x F) 31.60. For other explanation Please see Table 1.

Table 6: Nitrification in soil untreated or treated with normal agricultural rate of pesticides at different intervals of time, 1997

| Days | Sampling time | Control field | Test field | Farmer field | Mean |
|---|---|---|---|---|---|
| | | ($\mu$g $(NO_2+NO_3)$-N g$^{-1}$ dry soil | | | |
| 0 | Sowing | 20.95 defgh | 16.49 fgh | 27.20 bcd | 21.55 C |
| 50 | BPA | 23.96 cdef | 14.93 gh | 21.81 cdefg | 20.23 C |
| 146 | AAPA* | 18.90 efgh | 25.78 bcde | 13.53 h | 19.47 C |
| 238 | Harvest | 27.75 bcd | 25.01 bcde | 27.65 bcd | 26.80 B |
| 298 | Post harvest | 29.70 bc | 32.21 b | 42.94 a | 34.88 A |
| Mean | | 24.25 A | 22.88 A | 26.62 A | |

* 2 days after all pesticide applications. LSD (5%): For Sampling time (S) 4.19; for fields (F) ns; for interaction (S x F) 7.26. LSD (1%): for sampling time (S) 5.89; for interaction (P x F) 10.08. For other explanation see Table 1.

Table 7: Nitrification in soil untreated or treated with normal agricultural rate of pesticides at different intervals of time, 1998

| Days | Sampling time | Control field | Test field | Farmer field | Mean |
|---|---|---|---|---|---|
| | | ($\mu$g $(NO_2+NO_3)$-N g$^{-1}$ dry soil | | | |
| 0 | Sowing | 71.60 e | 67.63 e | 104.17 d | 103.32 C |
| 51 | BPA | 42.55 f | 74.80 e | 103.00 d | 72.84 D |
| 137 | 2 days APA* | 169.20 ab | 166.27 ab | 160.12 bc | 165.18 B |
| 210 | 73 days APA | 62.77 ef | 60.51 ef | 80.91 de | 68.06 D |
| 251 | Post harvest | 174.83 ab | 186.14 a | 177.15 ab | 174.37 A |
| Mean | | 117.50 AB | 111.07 B | 125.29 A | |

* 2 days after all pesticide applications LSD (5%): For sampling time (S) 17.95; for interaction (S x F) 31.12. LSD (1%): for sampling time (S) 12.94; for fields (F) 10.02; for interaction (S x F) 22.43. For other explanation Please see Table 1.

Table 8: Nitrification in soil untreated or treated with normal agricultural rate of pesticides at different intervals of time, 1999

| Days | Sampling time | ($\mu$g $(NO_2+NO_3)$-N g$^{-1}$ dry soil |
|---|---|---|
| -32 | Before sowing | 204.07 |
| 0 | Sowing | 201.95 |
| 44 | BPA | 184.50 |
| 109 | 2 days APA | 212.90 |
| 146 | Before harvest | 214.83 |
| 177 | Harvest | 210.12 |
| 216 | Post harvest | 207.84 |
| | Mean | 205.17 |

LSD (5%): For treatments; ns

results of stimulation with chloropyrifos alongwith other pesticides were observed in present study. Schuster and Schroeder, (1990b) studied side effects of sequentially and simultaneously applied pesticides on non-target soil organisms in laboratory. They found that nitrification was highly altered upon overdose and completely stopped, indicating that specific function of microflora may be completely arrested. Inhibition of nitrification however, depends upon the organic matter content of the soil. Present results are also supported by Tu (1995), that no inhibitory effects were observed with amitaz, cyfluthrin, imidacloprid, tebupirimphos and azetec on nitrification of the ammonium from soil organic nitrogen during the 2-week study. The greater mineralization of nitrogen following insecticide BHC, phorate, carbofuran, and fenvalerate, application brought a significant increase in nitrate in soil (Das and Mukerjee, 1994) and this remained more prominent upto 30$^{th}$ day, followed by a steady decline upto 60th day except with BHC and phorate both of which caused a progressive increase in $NO_3$-N until the 45th day. Fenvalerate showed inhibition at field rates of application on nitrification, which is contradictory to present results. These finding indicated that insecticides probably induced the growth and activity of nitrifying microorganism which in turn, release large amounts of $(NO_2+NO_3)$-N into the soil from the $(NH_4)_2SO_4$ because the substrate for nitrifying bacteria is the ammonium ions, a supply of this ion is the first requirement for nitrification (Tu, 1995).

Only one carbamate insecticide, carbosulfan was sprayed which showed no effect individually as well as in combination with other pesticide applications on nitrification. Wainwright and Pugh (1973) found that very low concentration of the fungicide captan, thiram and verdasan stimulated the nitrifiers but only high doses were inhibitory and ammonium oxidation was more susceptible than nitrite oxidation. The fungicide, benomyl, was reported to inhibit nitrification but its degradation product, methyl 2-benzimidazole carbamate, was not inhibitory. Therefore, the breakdown of benomyl would eliminate any inhibition of nitrification.

Data regarding nitrification in samples collected at sowing, before pesticide applications, after all pesticides applications, at harvest and at post harvest is shown in Tables 6 and 8. Maximum nitrification was carried out in soil samples collected at post harvest stage in first year (1997) and 2 days after all pesticides applications in second year (1998). Same nitrification was observed from sowing till all pesticides application as well as in all the fields in the first year of study. Nitrification in soil samples of second year study indicated that nitrification before and 73 days after all pesticide applications was quite similar but all other intervals differ significantly. Samples collected at the same sampling time showed no variation in nitrification at different intervals of time during the whole season (Table 8). The results of the present study clearly indicated that the application of different pesticides in cotton agroecosystem at their recommended normal agricultural rates of application reach the soil in only very small quantities which may affect the microbial processes like nitrification. These effects were short-lived and there was recovery of microflora and its functions were found later on.

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