Dynamics of clopyralid herbicide during composting in small composting experiment units

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Owing to its long persistence in the environment, residual clopyralid in compost has emerged as a problem worldwide. We mixed 9 kg by weight of dairy cow manure and 3 kg by weight of straw, and a clopyralid solution was added to each of three 3-kg aliquots until the clopyralid concentrations were 0, 6.7, and 20 mg/kg, respectively. To understand its fate during composting, the samples were composted in small composting experiment units for 77 days. Samples were collected at 7, 13, 18, 25, 32, 42, 57, and 77 days; each time, the composts were turned. Clopyralid was concentrated in the compost during primary fermentation and slowly decomposed during secondary fermentation. The ease of elution of clopyralid during composting shows that drainage must be managed during the composting of materials that might contain clopyralid. © Pesticide Science Society of Japan

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Introduction

In the late 1990s, some compost products damaged crops and gardens in the USA.13) Investigation of the cause revealed residual herbicide in the compost.22) The herbicide was clopyralid (3,6-dichloro-2-pyridinecarboxylic acid), a product of Dow AgroSciences. In response, various research institutes and agencies, including Dow AgroSciences, offered countermeasures against clopyralid contamination of compost.3–11) Although clopyralid levels in compost tended to decline as a result, residual clopyralid was still reported.9,12)

Clopyralid is used in many countries.13) Residual clopyralid in compost has also been detected in New Zealand.14) Although damage similar to that reported in the USA has yet to be reported elsewhere, some countries have drawn attention to the risks.15,16)

Increasing livestock densities in Japan began to cause serious problems with the disposal of excrement, so in 1999, the government restricted open-air composting by livestock farmers to decrease the leaching of nitrates into the environment. Crop damage due to clopyralid began to appear in the early 2000s, even though clopyralid is not registered for use in Japan.17) Compost made with livestock manure from dairy cows fed on imported hay contaminated with clopyralid was the source of the damage, because the restriction on open-air composting also decreased the leaching of residual clopyralid, which is poorly decomposed.

Clopyralid is an auxin-like compound used for killing broadleaf weeds and is not metabolized in plants.18) The behavior of clopyralid in Canada thistle (Cirsium arvense) has been extensively investigated, and the uptake, absorption, translocation, accumulation, and foliar activity of clopyralid have been elucidated.19–21)

Not only broadleaf weeds but also broadleaf crops are affected at clopyralid concentrations of <10 ppb in soils and composts.12) Therefore, the persistence of clopyralid in the environment can be problematic. Reported half-lives of clopyralid vary widely. The half-life is stated to be from 14 to 56 d in German guidelines and from 2 to 94 d in US guidelines.18) That in clay, clay loam, and sandy loam soils ranged from 10 to 47 d under incubation at 10 to 30°C for 84 d.22) In the ground, clopyralid is mainly decomposed by microorganisms,23) and its half-life is shorter in unsterilized soil than in sterilized soil.24) When clopyralid was sprayed onto various organic substrates (wheat straw chop, high moor peat, pine wood sawdust), 64 to 73% decomposed within the first 30 d.25) However, according to the EPA, clopyralid can persist in soil with a half-life of up to 11 months.26) In activated sludge, only 25% of added clopyralid had decomposed after 66 d.27) In composted turfgrass clippings turned regularly, the concentration of clopyralid declined from 32 ppm to <1.4 ppm after 365 d.28) A study by Washington State University concluded that a waiting period of up to 1 year after

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the application of clopyralid could be necessary for treated grass clippings to be safely used in compost.\textsuperscript{29} As judged from the above studies, clopyralid decomposition in the environment depends on the initial concentration, soil temperature, soil moisture, soil type, climate, quality of organic matter (OM), activity of microorganisms, and other factors. Therefore, it is important to estimate the rate of clopyralid decomposition during composting in Japan.

Clopyralid passes through the body unchanged; rats excrete it rapidly in urine, and fecal elimination is a minor path.\textsuperscript{30} Urinary excretion data showed that ingested clopyralid is rapidly and nearly completely absorbed, and that excretion is also rapid; >90\% within 32 hr, mainly in urine.\textsuperscript{31} Metabolic studies suggest that clopyralid is excreted rapidly and unchanged in the urine of ruminants and poultry.\textsuperscript{32} Judging from the above, after imported hays containing clopyralid were fed to dairy cows, almost all clopyralid was excreted in the urine. Then because compost is made from feces mixed with urine, the dynamics of clopyralid in the composting process can be estimated by adding clopyralid solution to raw materials for composting.

In estimating compost quality, maturity is best assessed with plant growth bioassays.\textsuperscript{33} Although a variety of tests have been proposed for the assessment of compost maturity, several authors have concluded that using a single parameter as a maturity index is insufficient.\textsuperscript{34} Stability is an important aspect of compost quality\textsuperscript{35} and relates to the degree to which the OM has been stabilized during composting.\textsuperscript{36} Because stability can be defined numerically, this measure is more appropriate for estimating the compost condition. Laboratory tests of the compost respiration rate are used to assess compost stability as a microbiological parameter\textsuperscript{37}; respiration is measured as either the rate of CO\textsubscript{2} evolution or the rate of O\textsubscript{2} consumption.\textsuperscript{38,39} As clopyralid is mainly decomposed by microorganisms, we used the rate of O\textsubscript{2} consumption as an indirect measure of compost stability and the rate of decomposition of raw materials as a direct measure.

Clopyralid is highly water soluble (7.85 g/L in distilled water, 118 g/L at pH 5, 143 g/L at pH 7, 157 g/L at pH 9; 20°C\textsuperscript{40}). More than 99\% was dissipated by 80 to 90 d after a 1:1 mixture of clopyralid and picloram was applied at 0.56 kg a.i./ha in the compost, we mixed 9 kg of dairy cow manure (70–90\% moisture) and 3 kg of comminuted straw (10–20\% moisture). Then as much as 0.31\% of the applied clopyralid was carried in drainage water from the irrigation district and released into the South Saskatchewan River.\textsuperscript{41} It was subsequently detected in 15 reservoirs in the Northern Great Plains of North America, and was identified as a contaminant in drinking water drawn from these reservoirs.\textsuperscript{42} Thus, because of its high leaching potential in the environment, clopyralid is likely to be eluted during composting, although this was not reported in any of the previous studies.

To support efforts to decrease clopyralid concentrations in compost made in Japan, we designed this study to estimate the dynamics of clopyralid during composting and to elucidate the characteristics of its elution from compost.

### Materials and Methods

1. **Composting using small composting experiment units (SCEUs)**

We conducted the composting experiments in small composting experiment units (SCEUs; Kaguyahime; Fujihira Industry Co., Ltd., Tokyo, Japan; Fig. 1). The SCEU is a cylinder (253 mm diameter×700 mm height) made of stainless steel, with a lid sloping at 45°. Its maximum effective capacity is 12.3 L. It collects water drained from the compost at the bottom and water condensed from evaporation at the top. It has an aerator for accelerating aerobic fermentation and a data logger for recording internal and external temperatures during composting. Each SCEU is operated inside in a wooden heat-retention box.

#### 1.1. Experiment 1: Composting with different initial clopyralid concentrations

The experimental procedures are shown in Fig. 2. To prepare the compost, we mixed 9 kg of dairy cow manure (70–90\% moisture) and 3 kg of comminuted straw (10–20\% moisture). Then a clopyralid solution was added to each of three 3-kg portions...
to give final concentrations of 0, 6.7, and 20 mg/kg. We adjusted the moisture content in each portion (determined from the difference between fresh weight and oven-dry weight) to 53.6% (within the ideal range) with distilled water. After thorough mixing, each portion was placed in a SCEU for composting. During composting, samples of a few grams each were collected at 7, 13, 18, 25, 32, 42, 57, and 77 d, when the composts were turned. In each SCEU, 1.6 L of distilled water was added to balance water loss during the 77 d. The samples were analyzed for clopyralid concentration, water content, rate of OM decomposition, and rate of oxygen consumption.

1.2. Experiment 2: Composting with different initial moisture contents
We mixed 5 kg each of fresh and dried dairy cow manure without straw, which retains moisture. To three 2.5-kg portions we added 0, 1, or 2 L of distilled water, giving respective moisture contents of 49.6%, 60.7%, and 65.3%. Then we added a clopyralid solution to a final concentration of 10 mg/kg. After thorough mixing, the portions were composted for 40 d. Samples of compost, condensed water, and drained water were collected at 4, 11, 18, 25, and 40 d, when the composts were turned. Distilled water was not added this time. The samples were analyzed as in experiment 1.

2. Analysis of clopyralid in compost
Clopyralid (99.0% purity) was obtained from EQ Laboratories Inc. (Atlanta, GA, USA). Adsorption experiments were carried out using a batch equilibration procedure with five concentrations (1, 3, 5, 10, and 20 mg/L) of clopyralid in identical compost samples that did not otherwise contain clopyralid. In a 100-mL glass beaker, 10 g of compost in 50 mL of 0.01 M CaCl₂ was shaken for 1 hr on a reciprocal shaker, and the suspension was then suction-filtered through No. 6 filter paper (Advantec, Tokyo, Japan). The equilibrium concentration of clopyralid in each extract was determined by high-performance liquid chromatography (HPLC) on a GL Sciences (Tokyo, Japan) Inertsil ODS-3 column (150 mm length × 4.0 mm i.d.) at 1 mL/min with elution by 1:6 (v/v) acetonitrile–1% acetic acid and UV detection at 254 nm. The quantification limit was 0.1 mg/L. The amounts of clopyralid adsorbed in the composts were estimated as the difference between the initial and final equilibrium concentrations. Adsorption isotherms were calculated using the Freundlich equation from the amount of adsorption and the equilibrium concentration in the extracts. Then we similarly analyzed the compost samples from experiments 1 and 2 in triplicate, and calculated the amounts of adsorbed clopyralid from the adsorption isotherms. The amount of decomposed clopyralid was estimated as the difference between the amount added and the amounts adsorbed on compost and dissolved in extraction.

3. Measurement of rate of OM decomposition
By using ignition loss method at 550°C for 3 hr, OM contents were measured in the compost samples. The rates of OM decomposition were calculated based on the equation:

\[
\text{Rate of OM decomposition (\%) } = \left( \frac{W_{di} - W_{d0}}{W_{d0}} \right) \times 100
\]

where \(W_{d0}\) = the initial compost dry weight, and \(W_{di}\) = the compost dry weight at time \(i\).
4. Measurement of activity of aerobic microorganisms during composting

To estimate compost maturity, we measured the oxygen consumption rates of compost samples, using a compost maturity tester (Compotester; Fujihira Industry, Tokyo, Japan). We placed 50 g of compost in a 500-mL stainless steel pot, which was then placed in the incubator of the tester. After incubation at 35°C for 30 min, the oxygen consumption rate was measured at the same temperature during the next 30 min. The results are presented as mg/min/kg of compost.

Various OMs were included in the compost raw materials, and the behavior of each OM was not homogeneous during composting. The rate of OM decomposition represents an average rate of whole compost raw materials during composting. Whereas, the oxygen consumption rates were used as an indicator of easily biodegradable OM in compost raw materials.

5. Experiment 1 (continued): Elution of clopyralid from compost

We conducted elution experiments to estimate the degree of clopyralid elimination from composts prepared with initial concentrations of 6.7 and 20 mg/kg. We loaded 500 g of 77-d compost onto 25-cm-diameter strainers and eluted them with 1 L of distilled water per day over 4 successive days either by dripping or all at once (equivalent to about 20 mm of precipitation per day). The eluted solutions were fully collected, and their volumes and clopyralid concentrations were determined to calculate the amounts of clopyralid eluted.

Results and Discussion

1. Decomposition of clopyralid during composting

During the 77 d of composting, the rates of decomposition of the OM in the compost were 65% of dry weight (0 mg/kg clopyralid), 68% (6.7 mg/kg), and 61% (20 mg/kg) (Fig. 3). All treatments showed inflection points at around 20 d. These inflection points divided the composting process into primary and secondary fermentation stages. The composting process can be divided into primary and secondary fermentation stages. We defined primary fermentation as the initial decomposition of the most easily decomposable OM, with a rise of temperature to >60°C, followed by the decomposition of most of the rest of it with turning and the addition of air and water, with a fall of temperature to <50°C. We defined secondary fermentation as the subsequent slow decomposition of the resistant OM. No water drained from the SCEUs in this experiment. Condensed water samples collected at every sampling date had no detectable clopyralid.

We plotted the concentrations of clopyralid in compost on a dry-weight basis during the primary and secondary fermentation stages (Fig. 4). Although the concentration decreased during primary fermentation at the higher rate of clopyralid, it increased during secondary fermentation at both rates, and faster at the higher rate. These results suggest that the concentration of clopyralid reflects the difference between the rate of the OM in the compost decomposition and the rate of clopyralid decomposition.

To correct for changes in the rate of OM decomposition, we divided the rate of clopyralid decomposition by the rate of OM decomposition:

$$\frac{(C_0W_{i0} - C_iW_{i0})}{(W_{d0} - W_{di})/W_{d0}}$$

where $C_0=$ the initial clopyralid concentration, $W_{i0}=$ the initial compost raw weight, $C_i=$ the clopyralid concentration at time $i$, $W_{i0}=$ the compost raw weight at time $i$, $W_{d0}=$ the initial compost dry weight, and $W_{di}=$ the compost dry weight at time $i$. The ratio decreased rapidly until around 20 d and then increased slowly until the end of composting (Fig. 5). The inflection points at around 20 d suggest that clopyralid was much more resistant to
decomposition than the OM during primary fermentation, and gradually became more labile during secondary fermentation, that is, after the microorganisms had consumed the easily bio-
degradable OM. A ratio of 1 indicates that the rate of clopyralid decomposition is equal to the rate of OM decomposition. As all ratios were <1 (Fig. 5), we can conclude that clopyralid was more resistant to decomposition than OM during composting, although its resistance changed over the course of the composting process. Therefore, the prolonging secondary fermentation would help reduce the concentration of clopyralid in compost.

Because clopyralid is mainly decomposed biologically,23 we examined the relationship between its rate of decomposition and the rate of oxygen consumption, which was high when the compost contained a large amount of biodegradable OM, owing to heightened activity of microorganisms. The rate of decomposition was highest when the rate of oxygen consumption was <5 mg/min/kg (Fig. 6). This explains why the decomposition of clopyralid accelerated after the decline in aerobic microorganism activity (Fig. 5).

2. Elution of clopyralid from compost by watering
We measured how much of the remaining clopyralid could be eluted from the compost. Watering eluted a greater proportion at a higher concentration of remaining clopyralid, with no apparent difference between dripping and all-at-once watering patterns (Table 1). It would be useful to wash out clopyralid from compost by exposure to rainfall, but open-air composting by livestock farmers is now prohibited in Japan. Instead, farmers might be able to collect water for washing on site.

3. Effect of the initial concentration of clopyralid and the initial moisture of compost
The concentration of clopyralid had no apparent effect on the rate of oxygen consumption (Fig. 7a). Thus, the small amounts added did not affect the activity of aerobic microorganisms. In contrast, the rate of decomposition increased with increasing initial moisture content at the first turning; however, the rate of oxygen consumption was the same in all samples (Fig. 7b). The rate of decomposition increased between the first and second turnings and the rate of oxygen consumption at the second turning decreased with increasing initial moisture content. This pattern suggests that low moisture content restricts the activity of aerobic microorganisms, thus, limits the decomposition of biodegradable OM. This phenomenon affected the amount of water that was generated by the decomposition of OM during composting.

Throughout the composting period, the volume of condensation tended to increase in proportion to the initial moisture con-

![Fig. 5. Time-course of the ratio of the rate of clopyralid decomposition to the rate of OM decomposition during the composting period.](image)

![Fig. 6. Relationship between the rate of clopyralid decomposition and the rate of oxygen consumption.](image)

![Table 1. Amounts of clopyralid eluted from compost by different watering methods (experiment 1)](table)
The greatest amounts of eluted water came from compost with an initial moisture content of 60.7%. No water eluted from compost with initial moisture contents of 49.6% by 18 d or of 60.7% and 65.3% by 25 d; the difference was due to differences in the rate of OM decomposition and moisture content. Although no clopyralid could be detected in any of the samples of condensed water, as in the previous experiment, clopyralid was detected in all of the samples of eluted water. Although the total amounts of eluted water varied, the total amounts of clopyralid eluted in the water increased with increasing moisture content. Lower amounts of eluted water were associated with higher concentrations of clopyralid in the water, although the pattern was not clear.

These findings show that it is necessary to deal with water eluted from compost in order to prevent its drainage into surrounding agricultural lands when the raw materials for compost might include clopyralid.

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