Effects of Plant Residue, Root Exudate and Juvenile Plants of Rapeseed (*Brassica napus* L.) on the Germination, Growth, Yield, and Quality of Subsequent Crops in Successive and Rotational Cropping Systems

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Abstract: Double-low rapeseed cultivars that produce no erucic acid and have only a small amount of glucosinolates are widely used. However, the roots in mature plant residue and leaves and roots of these seedlings still contain a large amount of biologically active glucosinolates, and it is important to clarify the effect of rapeseed cultivation on the subsequent crops. We analyzed the biological activities of the tissues of double-low cultivar at harvest. The laboratory bioassays revealed that the seeds and roots of mature rapeseed plants contained some autotoxic components that were volatile and water-soluble. We also analyzed the effects of root exudates from growing rapeseed plants on rapeseed germination and growth using the stair-step method and found that the root exudates were autotoxic. In the field, we investigated the allelopathic effects of rapeseed plants on the growth, yield, and quality of the subsequent sunflower plant in the same field. The growth, yield and thousand kernel weight of sunflower were reduced by the preceding cultivation of rapeseed. There were many spontaneously germinated juvenile rapeseed plants in the sunflower field preceded by rapeseed cultivation. Analysis of the plant and soil nitrogen (N) indicated that the growth reduction of sunflower was not caused by the competition for N with the spontaneously germinated juvenile rapeseed plants. The main factors responsible for poor sunflower growth under field after the cultivation of rapeseed were suggested to be some non-volatile, water-soluble components produced by the roots of the spontaneously germinated juvenile rapeseed.

Key words: Allelopathy, Juvenile rapeseed, Non-volatile components, Root exudates, Sunflower.

RAPeseed (*Brassica napus* L.) contains glucosinolates in the parenchyma. Rapeseed varieties with low glucosinolate levels have been used because the hydrolyzed products of glucosinolates, such as thiooxazolidones and isothiocyanates, are toxic to animals (Astwood et al., 1949; Kawagishi, 1985). However, glucosinolates have been detected in the roots of the double-low cultivar ‘Kirariboshi’, which produces no erucic acid and has only a small amount of glucosinolate in the aboveground tissues (Yasumoto et al., 2010).

The hydrolyzed products of glucosinolates exhibit biofumigatory effects on soil-borne pests and diseases (Sarwar and Kirkegaard, 1998; Sarwar et al., 1998). The presence of glucosinolates in root tissues can reduce the growth of fungi and oomycetes (Smith and Kirkegaard, 2002). Larkin and Griffin (2007) reported that when rapeseed was grown as a green manure crop, it reduced powdery scab infections in a subsequent potato crop. Bellosta et al. (2004) reported that determining the type of glucosinolates present in the species is the first step in assessing their biofumigatory potential.

The allelopathic effects of rapeseed mulch incorporated into a field reduced the density and biomass of weeds (Boydston and Hang, 1995). Olezek (1987) reported that the volatile compounds from chopped *Brassica* spp. leaves had allelopathic effects. Exposure to the volatiles from the leaves delayed seed germination and decreased subsequent growth of these species. Peterson et al. (2001) reported that volatile isothiocyanates, one of the degradation products of glucosinolates, suppressed weed germination.
after the incorporation of turnip–rapeseed green mulch into the soil. They reported that the isothiocyanate produced in large amounts by the mulch suppressed weed germination.

Unlike the allelopathic effects of glucosinolates, the autotoxic effects of rapeseed tissues have not been reported. Autotoxic effects have been reported in other crops. For example, Bi et al. (2010) reported autotoxic inhibitory activity of the soil attached to commercially cultivated American ginseng. Kitazawa et al. (2005) reported that strawberry root exudates acted as a potent growth inhibitor. And Asao et al. (2008) reported that electrodegradation of the root exudates could alleviate the autotoxicity. Given that the glucosinolate content of rapeseed is well known, it is important to clarify whether rapeseed also possesses autotoxic as well as allelopathic effects.

Choesin and Boerner (1990) reported inhibitory effects of rapeseed on weeds as a result of nutrient competition. They found that the breakdown products of glucosinolates from *B. napus* decreased the availability of sulfur compounds to neighboring plants. Bowren and Pittman (1975) reported nutrient competition between the crops and spontaneously germinated rapeseed plants in the field. However, they did not discuss other effects of spontaneously germinated rapeseed plants, nor did they clarify whether suppression of the summer crop resulted from allelopathic effects or from nutrient competition.

In our laboratory, we are currently studying rotational cropping systems including rapeseed, wheat (*Triticum aestivum* L.), and sunflower (*Helianthus annuus* L.) for human consumption and to generate bio-diesel fuel. The growth of sunflower after the cultivation of rapeseed was poorer than after wheat under certain conditions (Okada et al., 2008). In the field of summer crops after winter rapeseed, juvenile rapeseed plants emerged densely. Because high glucosinolate levels have been detected in the roots of these juvenile plants (Yasumoto et al., 2010), the allelopathic effects may explain the observed growth inhibition.

The present study was designed to clarify the autotoxic and allelopathic effects of these seedlings in successive and rotational cropping systems. We investigated these effects in the laboratory and in the field. In the laboratory experiments, the inhibitory effects of freeze-powdered rapeseed tissue (seeds, stems, root) and the soil sampled from the rapeseed field were examined in glass bottles. The effects of water-soluble compounds were examined in open bottles and that of volatile compounds in closed bottles. Since the water-soluble compounds showed inhibitory activity, we also examined the effect of root exudate on the growth of rapeseed by the stair-step method. In the field experiments, the effect of spontaneously germinated juvenile rapeseed and other weeds on the growth of sunflower was examined.

### Materials and Methods

1. **Experiment 1: Bioassay of inhibitory effects of plant tissues, their water extracts and soil samples in closed and open bottles**

   **(1) Freeze-powdered plant tissue**

   The seeds, stems and roots of mature rapeseed (var. Kirariboshi) were sampled on 20 June 2006. They were frozen in liquid nitrogen, vacuum-freeze-dried (Laytant...
LFG-600C S2, Laytant, Kanagawa, Japan) and milled into a fine powder by the method of Yasumoto et al. (2010). The leaves and stems of wheat plants that contained no glucosinolates were also powdered for comparison. In the experiment to examine the effect of volatile compounds, 0.01 or 0.10 g dry weight of each powdered sample was placed in a 20 mL glass bottle (25 mm in inner diameter and 55 mm in inner depth), and to separate the sample from the seeds, a piece of aluminum foil with a wet paper towel on it was placed on the sample. Six seeds of rapeseed, Kirariboshi, were sown on the paper towel, and the bottle was sealed to retain the volatile compounds. In the experiment to examine the effect of water-soluble compounds, 0.1 g powder was placed in each bottle, and 1 mL of distilled water was added to each bottle. Six seeds were sown in each bottle without sealing the bottle (without cap), and all bottles were kept in darkness at 22°C. Three bottles were used for each treatment (Table 1).

(2) Water extract
To obtain the water extracts of the plant tissues, we suspended 0.5 g of the powdered seeds, stems and roots in 5 mL of water and shook them for 48 hr at 22°C. The suspension was passed through a filter paper (Whatman No. 5B), and diluted 10 times with distilled water. Then, 1 mL of these extracts was applied to a piece of filter paper (Whatman No. 5B), and diluted 10 times with distilled water. Then, 1 mL of the extracts was applied to a piece of filter paper placed in the bottle, and 6 seeds of rapeseed were sown on it. As the control, paper towel wetted with 1 mL of distilled water was placed in a glass bottle. Six seeds of rapeseed were sown on each filter paper or wet paper towel, and incubated at 22°C in the dark (Table 1).

(3) Soil samples
Rapeseed (Kirariboshi) was sown in rows on 27 October 2006, and grown in the field. Soil samples were collected from the row spacing zone on 23 February 2007, when the leaf number of rapeseed plant was about four, at a depth of 10 cm at five points. The mixed soil samples were air-dried dry weight of soil sample (air dried) in a 20 mL glass bottle, and passed through a 2-mm mesh sieve. To examine the effect of the volatile compounds, we placed 0.01 or 0.1 g powder was placed in each bottle, and 1 mL of distilled water was added to each bottle. Six seeds were sown in each bottle without sealing the bottle (without cap), and all bottles were kept in darkness at 22°C. Three bottles were used for each treatment (Table 1).

(4) Measurement
Germinated seeds were counted at 24-hr interval for 72 hr, and the lengths of hypocotyls and roots were measured a 72 hr after sowing (Table 1).

2. Experiment 2: Effect of rapeseed root exudate (Stair-step experiment)
The auto-toxic effect of the root exudates from rapeseed was examined by stair-step method (Bell and Koepppe 1972; Fuji et al., 1991) in a greenhouse. In this method, “server” pots with rapeseed plants were watered and the drainage water was supplied to “receiver” pots. In this system the drainage was regarded as containing the root exudate of rapeseed (Fuji et al. 1991).

In the experiment to examine the effects of the root exudate on germination, 10 rapeseed (Kirariboshi) seeds were sown on 25 April 2007 in the server pot containing 150 mL river sand and 2 g ammonium sulfate and watered to grow the plants. The receiver pot was prepared in the same way as server pots but rapeseed was sown on 15 May 2007 (20 d after sowing in server pots) and watered with the drainage from the server pots. The germination percentage in the receiver pot was examined at 24-hr intervals for 72 hr.

In the experiment to examine the effects of the root exudate on plant growth, 10 seeds of rapeseed were sown in the server pot on 21 May and in the receiver pot on 30 June. After germination, both pots were watered normally, but from 2 July, 150 mL of water was supplied to the server pot and the drainage was supplied to the receiver pot two times a day. The growth of the stems and roots was examined on 23 July (3 weeks after 2 July), and compared with those of the control plants that received the drainage from the server pots without rapeseed plants. These experiments were conducted using three replicates.

3. Experiment 3: Effects of spontaneously germinated rapeseed plants on the growth of sunflower in the field
(1) Field design
Field experiments were carried out in 2007 and 2008 in an upland field at the National Agricultural Research Center (Tsukuba City, Ibaraki, Japan). Sunflower seeds (‘Armarvirkj 3497’, a Russian cultivar) were sown in a 3 m × 01 m field in 2007 and in a 3 m × 03 m field in 2008. They were sown with 75 cm row spacing, and 25 cm plant spacing. Compound fertilizer that contained 8.4 g m⁻² each of N (as (NH₄)₂SO₄), P (P₂O₅), and K (K₂O) was uniformly broadcasted prior to planting in both years. The preceding winter crop in the field was the double-low rapeseed cultivar ‘Kirariboshi’. After sowing the sunflower, we found densely growing juvenile rapeseed plants germinated from seeds of the preceding rapeseed crop, but juvenile weeds such as tufted knotweed (Persicaria longiseta (De Brans) Køng) were also growing densely in the field. Based on these juvenile plants, we divided the site into three plots of equal area (3 m × 19 m) separated by a 2-m-wide gap. In the first plot, all weeds except for rapeseed plants were weeded. In the second plot, juvenile weeds except for tufted knotweed plants were weeded. In
the third plot, all weed plants were weeded (weed-free control) (Fig. 1). In 2008, the preceding winter crop in the field was wheat.

(2) Plant and soil samples

Sunflower seeds were sown in the three plots on 13 June in 2007 and on 27 May in 2008, at a depth of 2 to 3 cm following rapeseed and wheat cultivation in the previous winter. We analyzed the following parameters of the sunflower in five plants from each plot; stem length, head diameter, disk thickness, leaf number, and total N content of the leaf. Total N content was analyzed using a hand-held optical sensor (SPAD-502, Konica Minolta, Inc. Tokyo, Japan) after flowering time. The SPAD values were measured at the center of the youngest fully-expanded leaf in each plant and then averaged. Before the measurement, we examined the relationship between the total N in the sample leaves using 13 plants. We determined the total N in the sample leaves using an automatic NC analyzer (Sumigraph NC22F). Nitrate nitrogen (NO₃-N) and ammonium nitrogen (NH₄-N) were extracted with 50 mL of 2 mol L⁻¹ KOH solution using 5 g of air-dried soil (Keeney and Nelson, 1982). The corresponding concentrations were determined using colorimetry with an automatic analyzer (TRAACS, Bran + Luebbe, Norderstedt, Germany). The phosphate buffer–extractable organic nitrogen (PEON) was measured according to the method of Ogawa et al. (1989). For this extraction, we mixed 5 g of the air-dried soil sample with 25 mL of 0.067 mol L⁻¹ phosphate buffer, shook the solution for 1 hr and filtered it though No. 6 filter paper (Advantar, Toyo Roshi Kaisha, Osaka.

![Typical photographs showing the experimental plots.](image)

(A) Many rapeseed seedlings had germinated. (B) Many tufted knotweed (*Persicaria longiseta* (De Bruyn) Kitag.) seedlings had germinated. (C) All weed plants were removed, the weed-free control.

![Relationship between leaf SPAD values and the total N content of sunflower leaves.](image)

The contents of oil and total fatty acids and the fatty acid composition were determined by the Caviezel method (Pendl et al., 1998), using a B-820 gas chromatograph (Nihon Büch Co. Ltd., Tokyo, Japan). The fatty acid composition was calculated using the relative values of the peak areas in the chromatogram to calculate the percentage to the total fatty acid content.
Therefore, the PEON value was used as an appropriate indicator of levels of available nitrogen in soils. All measurements were conducted with three to five replicates.

4. Statistical analysis

The results are presented as means, standard deviations, and standard errors. All data were subjected to a one-way analysis of variance (ANOVA), with Tukey's multiple-range

| Bottle (sealed with a cap) | Experimental samples | Germination (%) |
|---------------------------|----------------------|-----------------|
| Closed                    | Rapseseed seeds      | 22.2 ± 5.6 c    |
|                           | Rapseseed stems      | 38.9 ± 5.6 c    |
|                           | Rapseseed roots      | 33.3 ± 0.0 c    |
|                           | Soil samples from the row spacing of rapeseed cultivation | 61.1 ± 5.6 b |
|                           | Comparison (Wheat leaves and stems) | 100.0 ± 0.0 a |
|                           | Control (no sample)  | 100.0 ± 0.0 a   |
| Open                      | Rapseseed seeds      | 22.2 ± 5.6 b    |
|                           | Rapseseed stems      | 88.9 ± 11.1 a   |
|                           | Rapseseed roots      | 44.4 ± 5.6 b    |
|                           | Soil samples from the row spacing of rapeseed cultivation | 100.0 ± 0.0 a |
|                           | Water extracts of rapseseed seeds | 27.8 ± 5.6 b |
|                           | Water extracts of rapseseed stems | 100.0 ± 0.0 a |
|                           | Water extracts of rapseseed roots | 38.9 ± 5.6 b |
|                           | Water extracts of soil samples from the row spacing of rapeseed cultivation | 88.9 ± 5.6 a |
|                           | Comparison (Wheat leaves and stems) | 94.4 ± 5.6 a |
|                           | Control (no sample)  | 100.0 ± 0.0 a   |

In a 20 mL glass bottle, 0.1 g of powdered sample or soil, or 0.1 g mL⁻¹ of water extract was placed. Values followed by different letters differ significantly (Tukey's test, P < 0.05). Values are means ± SE of three replicates.

| Bottle (sealed with a cap) | Experimental samples | Hypocotyle length (mm) | Root length (mm) |
|---------------------------|----------------------|------------------------|-----------------|
| Closed                    | Rapseseed Seeds      | 0.4 ± 0.4 c            | 1.0 ± 0.4 b     |
|                           | Rapseseed Stems      | 6.2 ± 2.0 bc           | 2.6 ± 1.0 b     |
|                           | Rapseseed Roots      | 1.0 ± 1.0 c            | 2.0 ± 0.5 b     |
|                           | Comparison (Wheat)   | 9.3 ± 1.6 b            | 9.1 ± 1.8 a     |
|                           | Control (no sample)  | 15.6 ± 1.4 a           | 10.8 ± 0.8 a    |
| Open                      | Rapseseed Seeds      | 1.4 ± 1.2 b            | 1.4 ± 0.5 b     |
|                           | Rapseseed Stems      | 3.5 ± 0.9 b            | 5.5 ± 0.3 a     |
|                           | Rapseseed Roots      | 0 b                    | 1.2 ± 0.5 b     |
|                           | Comparison (Wheat)   | 6.0 ± 2.3 ab           | 5.8 ± 1.6 a     |
|                           | Control (no sample)  | 11.3 ± 2.9 a           | 8.0 ± 1.4 a     |

In a 20 mL glass bottle, 0.1 g of powdered sample was placed, and the lengths of hypocotyl and root were measured at 72 hr after sowing. Values followed by different letters differ significantly (Tukey's test, P < 0.05). Values are means ± SE of three replicates.
Germination of rapeseed in the river sand supplied with rapeseed root exudate by the stair-step method. Values are means ± SD of three replicates.

●, rapeseeds that received the root exudates; ○, control (without root exudate). **; Significant at P < 0.01.

Table 4. Effects of root exudates from server pots with rapeseed plants on growth of the rapeseed plants.

| Server Pots | Plant Length (cm) | Root Length (cm) |
|-------------|-------------------|------------------|
| Rapeseed    | 5.6±0.6**         | 8.5±1.8**        |
| Cont.       | 7.8±0.9           | 11.8±0.5         |

**: Significant difference at P<0.01. Values are means ± SD of three replicates (pots).

Growth of sunflower plants after rapeseed cultivation in the field with juvenile rapeseed, juvenile tufted knotweed and without weeds.

| Juvenile plants | Flowering (date) | Maturity (date) | Stem length (cm) | Head diameter (cm) | Disk thickness (cm) | Leaf number | N content (%) |
|-----------------|-----------------|-----------------|------------------|--------------------|---------------------|------------|--------------|
| Rapeseed        | 31 July         | 2 October       | 170.0±4.9 b      | 15.4±1.1 b         | 2.5±0.1 b           | 28.7±0.9   | 2.7±0.7 b    |
| Tufted knotweed | 31 July         | 28 September    | 178.0±5.4 a      | 20.0±1.0 a         | 2.5±0.2 b           | 28.3±1.4   | 4.6±0.8 a    |
| Weed-free       | 31 July         | 30 September    | 185.7±8.6 a      | 18.0±1.5 a         | 3.2±0.2 a           | 29.6±1.2   | 5.5±0.9 a    |

Values followed by different letters differ significantly (Tukey's test, P<0.05). Values are means ± SD of three to five replicates.
test used to compare mean values when the ANOVA revealed a significant difference. We performed this analysis using version 11.0 of the Japanese SPSS software (SPSS Inc., Chicago, USA).

Results

1. Experiment 1: Bioassay in closed and open bottles

Table 2 shows the effects of freeze-powdered tissues (seed, stem and root) of rapeseed and soil samples and their water extracts on germination of rapeseed. The effect of freeze-powdered wheat tissue is shown for comparison. In the closed bottle, the seeds of rapeseed were placed separately from the powder to examine the effect of volatile compound, and in open bottles, the seeds were sown in the water with the powder, to examine the effect of water-soluble compounds.

In the closed bottles, germination of rapeseed was suppressed by all samples except for the wheat sample. In the open bottles, the germination was suppressed by powdered samples and water extract of the seed and root, but not or only non-significantly suppressed by powdered stems and soils and their water extracts. Then, the powdered stem and soil samples inhibited the germination of rapeseed in closed bottle but not in the open bottle, suggesting that they contained some volatile chemicals capable of suppressing germination. In open bottles, the water extract of powdered stem and soil did not suppress the germination, while they suppressed the germination in the closed bottle. This suggests that they have some water-soluble nonvolatile compounds capable of suppressing germination. The powdered seed and root suppressed germination in both closed and open bottles suggesting that they contain both volatile and water-soluble compounds capable of inhibiting germination.

Table 3 shows the effects of freeze-powdered plant tissues of rapeseed and wheat on the growth of rapeseed. The powdered samples of seeds and roots (0.1 g mL$^{-1}$ water) greatly suppressed the growth of the hypocotyl and roots at 72 hr after sowing in both closed and open bottles. The powdered stem also suppressed the growth of the hypocotyl and root, but the effect was weaker than that of the powdered samples of seed and root. Powdered tissues (leaves and stems) of wheat had no significant effect. Fig. 3 shows photographs of the rapeseed plants germinated in the closed and open bottles containing different amounts of powdered samples at 72 hr after sowing. The inhibitory effect of the powdered root of rapeseed was dose dependent, and was greater than that of the powdered wheat tissue.

2. Experiment 2: Stair-step experiment

Fig. 4 shows the effect of root exudate of rapeseed on germination of rapeseed examined by the stair-step method, and Table 4 shows the effect of the root exudate on the growth of rapeseed examined by the same method. Germination at 48 hr after sowing was greatly suppressed by applying the root exudate (Fig. 4). Plant length and root length of rapeseed were also significantly reduced by applying the root exudate (Table 4). Fig. 5 shows a photograph of the rapeseed seedlings supplied with and without root exudate in the stair-step experiment, which confirmed the result shown in Table 4.

3. Experiment 3: Effects of spontaneously germinated rapeseed plants on the growth of sunflower in the field

Fig. 1 shows the experimental field where sunflower plants were cultivated with and without weeding. This shows that there was no light competition between sunflower and rapeseed or tufted knotweed. Table 5 shows the growth of sunflower growing together with and without juvenile weeds. The flowering date was not affected by the presence of weeds, but the maturity date was delayed by the presence of juvenile rapeseed plants. The stem length and head diameter of sunflower was decreased significantly by the presence of spontaneously germinated juvenile rapeseed plants, but not by tufted knotweed. The disk thickness of sunflower was significantly decreased by the presence of either rapeseed or tufted knotweed. Leaf number was not affected by the weeds. However, the N content of the sunflower leaves, which was significantly correlated with the SPAD value (Fig. 2), was significantly decreased by the presence of juvenile rapeseed plants, but not significantly by tufted knotweed (Table 5). Table 6 shows the growth (dry weight) and nitrogen (N) absorption of juvenile rapeseed and tufted knotweed. N absorption by the tufted knotweed (21.2 g m$^{-2}$) was significantly higher than that by rapeseed (7.9 g m$^{-2}$). The dry weight of tufted knotweed m$^{-2}$ was significantly heavier than that of rapeseed. Table 7 shows the total and available N in the soils from the field with juvenile rapeseed, juvenile tufted knotweed, and without any weeds. The total and available N contents of the soil were not significantly influenced by the weeds growing on it (Table 7). However, the N content of sunflower leaf was significantly decreased by the presence of juvenile rapeseed plants, and not significantly by the presence of tufted knotweed (Table 5).

Table 6. Growth (dry weight) and nitrogen (N) absorption of juvenile rapeseed and tufted knotweed.

| Juvenile plants | Dry weight of juvenile plants (g m$^{-2}$) | N absorption of juvenile plants (g m$^{-2}$) |
|-----------------|------------------------------------------|------------------------------------------|
| Rapeseed        | 141.4 ± 6.01 b                           | 7.9 ± 0.3 b                              |
| Tufted knotweed | 243.0 ± 47.1 a                           | 21.1 ± 7.4 a                             |

Values followed by different letters differ significantly (Tukey’s $t$ test, P < 0.05). Values are means ± SE of three replicates.
The sunflower seed yield was significantly lower in the field with juvenile rapeseed than in the field with tufted knotweed and weed-free field (Table 8). The oil content of sunflower seeds (% and seed⁻¹) was not significantly influenced by the weeds, but the thousand-kernel weight was significantly decreased by the presence of rapeseed though not by tufted knotweed (Table 8). The oleic acid (18:1) content was significantly higher and the linoleic acid (18:2) content was significantly lower in sunflower grown in the field with rapeseed than in the field without rapeseed: the linolenic acid (18:3) content was not significantly decreased by the weeds (Table 8).

| Juvenile plants | Total N in the soil (g m⁻²) | Available N in the soil (g m⁻²) |
|----------------|-----------------------------|-------------------------------|
|                 | NO₃⁻N          | NH₄⁻N          | PEON          |
| Rapeseed        | 297 ± 19b      | 2.71 ± 0.35    | 0.75 ± 0.03   | 4.85 ± 0.12 |
| Tufted knotweed | 328 ± 11ab     | 2.87 ± 0.11    | 0.66 ± 0.02   | 4.86 ± 0.02 |
| Weed-free       | 350 ± 7a       | 2.83 ± 0.24    | 0.62 ± 0.01   | 4.88 ± 0.14 |

Values followed by different letters differ significantly (Tukey’s t-test, P < 0.05). Values are means ± SE of three to five replicates.

Discussion

In our previous research (Yasumoto et al., 2010) glucosinolates such as glucobrassicanapin (4-pentenyl glucosinolate) and gluconasturtin (2-phenylethyl glucosinolate) were detected in the freeze-dried leaves, stems and roots of ‘Kirariboshi’ rapeseed. These glucosinolates differed from the major glucosinolates in rapeseed (Ishida et al., 1995), which included progoitrin (2-hydroxy-3-butenyl glucosinolate) and gluconapin (3-butenyl glycosinolate). Because some glucosinolates were detected even in a double-low cultivar of rapeseed (Yasumoto et al., 2010), we hypothesized that the rapeseed plant residues might have an auto-toxic effect. To test this hypothesis, we first examined the effects of freeze-powdered samples of rapeseed plant tissues and soil samples collected from the row spacing zone of the field of rapeseed cultivation on seed germination. As a control, wheat plant that contained no glucosinolates was used. The powdered rapeseed seeds and roots clearly suppressed germination of rapeseed in both closed and open glass bottles showing that both of the volatile and water-soluble components inhibited seed germination. The suppressive effects increased with increasing amount of the powder (Fig. 3).

Table 7. The total and available N in the soils of the field with juvenile rapeseed, juvenile tufted knotweed and without weeds.

Table 8. The yield and quality of sunflower grown together with juvenile rapeseed, juvenile tufted knotweed and without weeds in 2007.

Table 9. The growth, yield and quality of sunflower grown in the field without any weeds in 2008, following wheat cultivation in the previous winter.
The growth of sunflower in the field was poor after rapeseed cultivation probably due to the presence of many juvenile rapeseed plants that had spontaneously germinated from seeds dropped during harvesting of the preceding crop. At first, we thought that the main factor responsible for the poor growth of the sunflower crop after rapeseed cultivation was competition for $N$. To test this hypothesis and to clarify whether suppression of the summer crop resulted from allelopathic effects or from nitrogen competition, we analyzed the $N$ contents of the plants and soils. The $N$ content of the leaf of sunflower in the field with spontaneously germinated rapeseed was significantly lower than that in the other fields (Table 5). The total soil $N$ was significantly lower in the field with rapeseed growing than in the weed-free field. However, there were no significant differences between the fields with rapeseed growing and that with tufted knotweed growing or between the field with tufted knotweed growing and the weed-free field in the available soil $N$ (Table 7). Though tufted knotweed absorbed $N$ more than the rapeseed (Table 6), it significantly affected neither the growth nor $N$ uptake of sunflower, but decreased disk thickness (Table 5). These results suggested that the suppressive effects of juvenile rapeseed plant on the growth and $N$ uptake of sunflower was greater than that of tufted knotweed.

Vera et al. (1987) reported that rapeseed and other Brassica species had phytotoxic effects on the growth of subsequent crops. They reported that the incorporation of rapeseed and Brassica biomass into the soil decreased the plant density in subsequent crops. However, Gary and Lincoln (1999) reported that the glucosinolate contents of $Brassica juncea$ L. tissues decreased rapidly after their incorporation into the soil. Only small quantities of glucosinolates remained after 6 d. Based on these reports, many researchers believed that the glucosinolates produced by the preceding crop would have degraded before the subsequent cropping in the same field, and that these compounds would not be responsible for poorer growth of the subsequent crop. We predicted that the seed and root tissues of the preceding rapeseed crop that were retained in the field under natural conditions would not affect subsequent crops.

However, in the fields we found many spontaneously germinated rapeseed, and detected a large amount of glucosinolate in these rapeseed plants (Yasumoto et al., 2010). Brown and Morra (1996) reported that the volatiles produced in intact roots of rapeseed inhibited the germination of lettuce seed by 70% on blotter paper (at a concentration of 5.5 g dry wt per 72.25 cm$^2$). The major glucosinolate in the roots was gluconasturtiin, (4.5 mol g$^{-1}$ dry wt). On this basis, the gluconasturtiin content of the sample would be 24.75 mol and the amount of gluconasturtiin per unit area would be roughly 3.4 mmol m$^{-2}$. The observed amount of gluconasturtiin in the seedling roots was 233 mmol m$^{-2}$. These results suggest that spontaneously germinated rapeseed seedlings affected the growth of succeeding crops by releasing glucosinolates from their roots into the rhizosphere.

Fuji et al. (1991) reported that root exudates from velvet bean ($Mucuna pruriens$ L.) had allelopathic effects on subsequent crops. They distinguished this allelopathic effect from the competition for light, nutrients, and water by rotating greenhouse experiments and step-stair experiments. Similarly, Yasuda et al. (1991) reported that the root exudates of common lambsquarters ($Chenopodium album$ L.) had allelopathic effects on crops cultured together. They confirmed that the effects were due to allelopathy rather than competition for light and nutrients by means of step-stair experiments. The results of the stair-step experiment in the present study also suggested that the rapeseed root exudates could suppress both germination and growth of subsequent crops. This effect therefore appears to be responsible for the poor growth of sunflower after rapeseed cultivation.

The quality of the sunflower seeds was also affected by weeds. The sunflower grown in the fields with juvenile rapeseed plants contained a significantly larger amount of oleic acid and significantly smaller amount of linoleic acid than those of sunflower grown in the field without rapeseed (Table 9). One possible explanation is that the growth and maturation of sunflower were suppressed (Table 5), and the yield and quality of its fatty acids were also influenced by the presence of rapeseed.

In the EU, the cultivation area of rapeseed has been increasing as a result of increased demand for manufacturing bio-diesel (Menguzzato and Rossetto, 2007). However, Vilhordo et al. (1985) reported that rapeseed had allelopathic effects on the subsequent growth of soybean ($Glycine max$ (L.) Merr.) during crop rotation between the two species. In Japan, the cultivation of oilseed crops such as rapeseed and sunflower is also expanding to support the production of bio-diesel. Thus, successive and rotational cropping systems for rapeseed and sunflower without autotoxic and allelopathic effects will be needed.

In this study, we analyzed the $N$ contents of plant leaves and soils. In the future, we will analyze the effects of juvenile rapeseed on other nutrients. We will also try to identify the biologically active substances in the root exudates and to find ways to minimize the numbers of seeds dropped from ruptured rapeseed capsules during harvesting. We hope to use this knowledge to develop an effective crop rotation system for rapeseed and sunflower.

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