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Title : Absorption and Translocation of ^{14}C-NTN 33893 in Eggplants and Rice Plants

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Contents

ABSTRACT

The absorption and translocation of NTN 33893 {imidacloprid (under application to ISO), 1-(2-chloro-5-pyridinylmethyl)-2-nitroiminoimidazolidine} in young eggplants and rice plants were investigated over a period of 8 days following application of [pyridinyl-^{14}C-methyl] NTN 33893 by painting to the aerial parts and addition to the nutrient solution. The behavior of ^{14}C-NTN 33893 was similar between the two plants. Following application to the aerial parts, ^{14}C penetrated into the plants and exhibited significant acropetal translocation. The distribution of ^{14}C applied to leaf blade, petiole (eggplants) and leaf sheath (rice plants) was almost restrictive to the applied leaf, especially to its marginal area, and was small in the other parts. In the case of stem application (eggplants) in which ^{14}C penetrated via the lower part of the plants than in the cases above, ^{14}C was distributed rapidly to all the upper parts of plants. The amount of unchanged NTN 33893 in leaf wash was greater in lower surface application than that in upper surface application. On the contrary, the rates of penetration and conversion were larger in upper surface application, suggesting the great contribution of photodegradation in foliar application of NTN 33893. Further, part of photodegradation products were assumed to possess more leaf-penetrability and volatility. In nutrient solution application, ^{14}C was absorbed via roots and translocated rapidly to the aerial parts, and accumulated to the leaf margins. Although NTN 33893 was metabolized in plant tissues after uptake via roots, the parent compound was still the main component of labelled residue in plants.

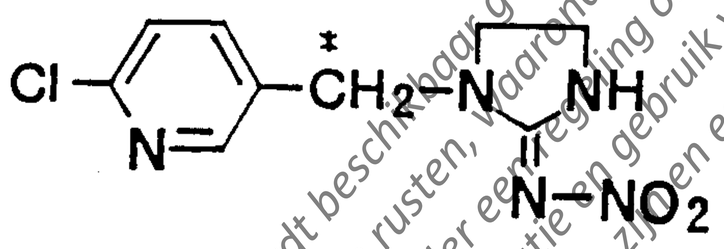
INTRODUCTION

NTN 33893 (imidacloprid (under application to ISO), 1-(2-chloro-5-pyridinylmethyl)-2-nitroiminoimidazolidine) is a new insecticide being developed by Nitokuno and Bayer AG for the control of plant hoppers and leaf hoppers in paddy field, aphids in upland, and so forth. In the study reported herein, the absorption and translocation of ^{14}C following application of ^{14}C-labelled compound to young plants (eggplants and rice plants) was investigated.

MATERIALS AND METHODS

1. Compound

[Pyridinyl-^{14}C-methyl] NTN 33893 was synthesized by [redacted] (Bayer AG) and had a specific activity of 5.58 MBq/mg (150.7 μCi/mg), a radiochemical purity of 99.2 % (at the application to eggplants) and 97.2 % (at the application to rice plants).



2. Detection and determination of ^{14}C

The amount of ^{14}C was determined as follows. Liquid samples were put into 20 ml glass or polyethylene vials and 5-10 ml of scintillation cocktail was added and mixed. Ready Gel was used for aqueous samples and silica gel scrapings, and Ready Organic was used for non-aqueous samples (both cocktails from Beckman). ^{14}C was counted by Beckman LS 5801 or LS 3801 liquid scintillation counter (LSC). Solid samples were combusted by Packard Tri-carb 306 sample oxidizer, followed by counting with LSC. CO_2-absorbent was Carbo-Sorb 7 ml and scintillation cocktail was Permafluor V 10 ml (both from Packard).

Radioactive zones on thin-layer chromatograms were detected by Berthold LB 284 TLC linear analyzer and autoradiography (ARG). XAR (Kodak) or Ix-150 (Fuji) X-ray films were used. ARG technique was applied also for the detection of ^{14}C in plants.

3. Preliminary test for stability and volatility of NTN 33893

3-1. Stability in organic solvents

^{14}C-NTN 33893 was dissolved in organic solvents (methanol, ethanol, acetone, acetonitrile, dichloromethane, ethyl acetate and toluene) at the concentration of 1 μg/ml. Each solution was pipetted into triplicate 10 ml Pylex® centrifuge tubes. One was placed under the laboratory light conditions (fluorescent lamp) others were covered with alminum foil and one was placed in the laboratory and the other in the refrigerator at 4°C. At several time intervals aliquots of each solution were analyzed by thin-layer chromatography (TLC, plate: Silica gel 60 F_{254}, 20 x 20 cm, 0.25 mm thick, developing solvent: toluene-ethyl acetate-methanol-acetic acid = 20+80+20+1, v/v).

3-2. Stability on silica gel thin layer

Methanol solution of ^{14}C-NTN 33893 was spotted on silica gel TLC plates (the same as above) at 2 x 10^4 dpm (ca. 60 ng)/spot. One plate was placed under the laboratory light conditions (fluorescent lamp), the other in the dark. The spots were developed (solvent: the same as above) after several time intervals and analyzed time-course conversion of the parent compound.

3-3. Stability in acid and alkaline solution

^{14}C-NTN 33893 was dissolved in 1N and 3N HCl, 6N-HCl-methanol (1:1) and 1N NaOH, respectively, at the concentration of 0.1 μg/ml. The solutions were refluxed at 100°C for 2 hr, followed by cooling in the air, neutralization and extraction with dichloromethane. The dichloromethane layer was analyzed by TLC (the same conditions as above).

3-4. Volatility in a rotary vacuum evaporator

Each 50 ml of ^{14}C-NTN 33893 solution of methanol, 80 % methanol, dichloromethane, acetone and ethyl acetate was put in 50 ml round-bottomed flask and the organic solvent was evaporated in a rotary evaporator at 40°C in vacuo. After removal of the organic solvent, ^{14}C in the solvent recovered in the trapping flask was counted.

3-5. Volatility in a lyophilizer

Small amount of methanol solution of ^{14}C-NTN 33893 was added in a petri dish (55 mm i.d.) and distributed uniformly to form thin layer of ca. 6 ng NTN 33893/cm^2. The petri dish was placed in a lyophilizer and processed at <0.1 Torr for 24 hr. ^{14}C in the trapping flask was counted. The thin layer of NTN 33893 covered with 5 % carboxymethylcellulose (CMC) was also tested for volatility.

4. Plants

Eggplants The seedlings at the 2-leaf stage were purchased from DIA-TOPY NOGEI LTD. and transferred immediately to water culture (nutrient solution: Green Plat®)*. The plants were allowed to grow for 11 days to reach the 4- to 5-leaf stage and then transplanted individually to foil-covered 200-ml Erlenmeyer flasks containing 200 ml of the nutrient solution. The plants were supported with cotton wool at the neck of the flasks. The test compound was applied after 2 days (Jun. 23, 1987), and the plants were cultivated, with daily supply of the nutrient solution, in the RI-greenhouse of the ESR building at the temperature of 25 ± 5 °C, humidity of 60 ± 20 % and under natural sunlight conditions.

Rice plants Rice seeds were surface sterilized in thiram 0.1 % + benomyl 0.1 % (WP) and soaked in water, and were sowed in a nursery box to be grown for 3 weeks. Then the seedlings were transferred to water culture (nutrient solution: Otsuka house®)**. After 6 days, on the application day (Jan. 18, 1988), the seedlings at the 4-leaf stage were transplanted individually to foil-covered 200-ml Erlenmeyer flasks containing 230 ml of the nutrient solution. The plants were supported in the same manner as eggplants. The plants were grown in the RI-greenhouse of the ESR building, at the temperature of 30 ± 5 °C, humidity of 60 ± 20 %, and at the photoperiod corresponding to the beginning of June (4:30 - 19:00) by the supplemental illumination using reflection sunbeam lamps (Toshiba).

* Green Plat® powder for nutrient solution No. 1 (N 10 %, P 8 %, K 23 %, Mg 4 %, Mn 0.1 %, B 0.1 %) 6.8 g and No. 2 (N 11 %) 4.6 g were dissolved in 10 l of water. pH: ca. 5.5

** Otsuka house® powder for nutrient solution No. 1 (N 10 %, P 8 %, K 23 %, Mg 5 %, Mn 0.1 %, B 0.1 %, Fe 0.18 %) 1.5 g and No. 2 (N 11 %, Ca 23 %) 1 g were dissolved in 1 l of water. pH: ca. 5.7

5. Application

5-1. Application solution

^{14}C-NTN 33893 was dissolved in ethanol to prepare the following 2 kinds of application solution.

A: 100 ppm solution, 5.58×10^2 Bq/μl (3.35×10^4 dpm/μl), 50 % ethanol was used in the preparation of the solution for rice plants.

B: 500 ppm solution, 2.79×10^3 Bq/μl (1.67×10^5 dpm/μl)

5-2. Application to eggplants

Balance study and fractionation study

Site of application	Method	Number of plants	
		Balance	Fractionation
Foliage leaves			
Upper Surface	Painting all over the 2nd & 3rd leaves, Solution A x 20 μl	8	2
Lower Surface		8	2
Nutrient solution	Mixing, solution B x 30 μl	8	2

ARG study

Site of application	Method	Number of plants
Foliage leaves		
Upper Surface	Painting on the basal part of the 2nd leaf, Solution A x 3 μl	2
Lower Surface		2
Stem	Painting just above the cotyledon, Solution A x 3 μl	2
Petiole	Painting on the upper petiole of the 2nd leaf, Solution A x 3 μl	2
Nutrient solution	Mixing, Solution B x 10 μl	2

5-3. Application to rice plants

Balance study and fractionation study

Site of application	Method	Number of plants	
		Balance	Fractionation
Leaf blade	Painting all over the 2nd & 3rd leaves(both surfaces), Solution A x 20 μl	8	2
Nutrient solution	Mixing, solution B x 30 μl	8	2

ARG study

Site of application	Method	Number of plants
Leaf blade	Painting on the central part of the 3rd leaf, Solution A x 3 μl	2
Leaf sheath	Painting on the upper part of the 3rd sheath, Solution A x 3 μl	2
Nutrient solution	Mixing, Solution B x 10 μl	2

6. Sampling and analysis

6-1. Balance study

Both eggplants and rice plants were sampled by 2 plants each at 1, 2, 4 and 8 days after application. In the case of leaf-applied plants, applied leaves were washed with ethanol (eggplants: 40 ml/leaf, rice plants: 30 ml/leaf) using pasteur pipettes and the washing solutions were collected. The roots were rinsed with water and the rinsings were mixed with the nutrient solution. The washed leaves, the other aerial parts, the roots and the nutrient solution were separated from each other and measured for weights or volumes. The plant materials were allowed to dry in the air. In the case of nutrient solution-applied plants, the roots were first washed with water. The aerial parts, the roots and the nutrient solution (including root rinsings) were processed in the same manner as above. The dried plant materials were cut into small pieces and combusted for the determination of ^{14}C. Aliquots of the leaf wash and the nutrient solution were counted for ^{14}C.

6-2. Fractionation study

All samples were collected at 8 days after application. The applied leaves were washed in the same manner as 6-1. The roots were rinsed with water. The samples were divided into the aerial parts, the

roots and the nutrient solution (including root washings). The aerial parts were pulverized in liquid nitiogen and extracted in the following manner. Eggplants were submerged in methanol and kept at -20°C until extraction, when methanol was added at the ratio of 10 ml/g sample and homogenized by Polytron® extractor for 2 min. The extract and the residue were separated by centrifugation at 9000 rpm for 15 min. The residue was further extracted likewise using 80 % methanol (twice). Rice plants were submerged overnight in 80 % methanol at the ratio of 10 ml/g sample and then extracted by ultrasonication (3 min) and shaking (30 min). The extract and the residue were separated by centrifugation at 3500 rpm for 10 min, and the residue was further extracted likewise by ultrasonication and shaking (twice). The three extracts of the respective plants were each collected and methanol was removed in a rotary vacuum evaporator at 40°C. The remaining aqueous solution was extracted with equal volume of dichloromethane 3 times (the two phases were separated by centrifugation at 3000 rpm for 5 min) to fractionate ^{14}C into dichloromethane layer (organo-soluble fraction) and aqueous layer (water-soluble fraction). An aliquot of the nutrient solution was extracted with dichloromethane and fractionated in the similar manner as the extract of the aerial parts. The fractions and leaf washes were each counted for ^{14}C.

The leaf washes and the dichloromethane fractions were then subjected to TLC to quantify the parent compound. The samples (ca. 2-5 x 10^4 dpm) and the standard solution of NTN 33893 were applied individually on TLC plates (silica gel 60 F_{254}, 5 x 20 cm, 0.25 mm thick, Merck) and developed by toluene-ethyl acetate-methanol-acetic acid (20+80+20+1, v/v). The radioactive zones were detected by TLC linear analyzer and ARG. The spots which had the identical Rf value as the standard NTN 33893 (UV-detection) were scraped into counting vials and counted for ^{14}C.

6-3. ARG study

The plants were sampled at 1- and 8-day, 1 plant each, and were spread between paper to prepare pressed plants. The roots of the nutrient solution-applied plants were rinsed in water before

processing. The pressed plants were mounted on pasteboards and exposed to X-ray films for ARG.

RESULTS AND DISCUSSION

1. Stability and volatility of NTN 33893

The results of the preliminary test are summarized in Table 1. In organic solvents and on silica gel thin layer, NTN 33893 was stable in the dark. However, degradation products were formed under the irradiation of fluorescent lamp. NTN 33893 was quite unstable in strongly basic solution, in contrast with in strongly acid conditions to which the compound was almost stable. The operation neither with rotary vacuum evaporator nor with lyophilizer caused volatilization of this compound. These results indicate that NTN 33893 is relatively stable in the course of extraction and analysis under usual laboratory conditions provided proper consideration is given such as shielding against light.

2. Absorption and translocation of ^{14}C in eggplants

2-1. Leaf blade application

The balance of ^{14}C following leaf blade application is shown in Table 2 (upper surface application) and Table 3 (lower surface application). In both applications more than 98 % of the retrieved ^{14}C was found from leaf wash and washed leaves at every sampling time, and only small amount was detected in the other parts. This suggests little translocation of the foliar-applied ^{14}C to the other plant parts. ARG (Fig. 1) clarified the distribution of ^{14}C being restricted almost in the applied leaf and its petiole following both applications. Only weak darkness was observed in a portion of non-applied plant parts on 1-day ARG. This information supports the suggestion from the balance study stated above. The marginal part of the leaf was darkened more intensively on 8-day ARG. All these findings indicate that the foliar-applied ^{14}C was translocated upward, and downward movement was small, resulting in the limited distribution of ^{14}C within the treated leaves.

The rate of penetration of ^{14}C into leaf tissues can be indicated by the balances of ^{14}C in leaf wash and washed leaves. Penetrated

amount of upper surface-applied ^{14}C increased time dependently, and at 8-day ca. 30 % of ^{14}C was recovered from leaf wash (not penetrated ^{14}C), ca. 55 % from washed leaves (penetrated ^{14}C). In lower surface application, the amount of ^{14}C not penetrated was 60 %, penetrated was 35 % at 1-day and the proportion was almost the same at every following sampling time.

Discrepancy between applied surfaces was noted also in the fractionation study (Fig. 2). The proportions of the parent compound in leaf wash at 8-day were 24.1 % (7.9 % of the dose) in upper surface application and 86.2 % (do. 58.8 %) in lower surface application. This difference seems to be attributable to photodegradation on the leaf surfaces, for more intensive degradation is expected to have occurred on the upper surface which was exposed to more irradiation of light than the lower surface. The proportions of the parent compound in the aerial parts after rinsing were remarkably smaller than those in leaf wash, being 7.6 % (3.5 % of the dose) in upper surface application, 15.8 % (do. 4.3 %) in lower surface application. This suggests the more penetrative nature of part of the photodegradation products than the parent compound, which is supported by the fact that the degree of photodegradation was upper surface > lower surface and the amount of ^{14}C penetrated was also upper surface > lower surface. The distribution pattern of ^{14}C in three fractions was similar between two applications as shown in the figure. Although the recovery of ^{14}C was almost quantitative in lower surface application (>96 %), that in upper surface application decreased gradually (87 % at 8-day). The results leads to the assumption that part of the photodegradation products can be volatile. The residual amount of the parent compound in whole plants 8 days after application was 11.4 % (upper surface application) and 63.1 % (lower surface application) of the dose.

2-2. Stem application and petiole application

The translocation of ^{14}C applied to stem and petiole was investigated using ARG technique alone. Following stem application (Fig. 3), only small amount of ^{14}C was detected in part of lower

stem and cotyledon, and most ^{14}C exhibited upward movement resulting in the distribution in all the leaves on the upper parts. In the case of petiole application (Fig. 3), the translocation of ^{14}C was observed to the leaf blade of the applied petiole, part of upper and lower stem and non-applied petiole at 1-day. However, at 8-day the image of stem and non-applied petiole disappeared. The information on the translocation of the stem-applied ^{14}C can account for this, as ^{14}C once distributed to stem and non-applied petiole was then translocated upward and dispersed in the upper parts of the plants. The darkness on the marginal areas of the ^{14}C-distributed leaves was found more intensive than the other areas at 8-day after both applications, similarly to the case of foliar application. These findings indicates the intensive acropetal movement of the label from ^{14}C-NTN 33893 penetrated via stem and petiole.

2-3. Nutrient solution application

^{14}C applied to the nutrient solution was distributed in a balance as shown in Table 4. The plants gradually absorbed ^{14}C and 39 % of the dose was recovered from the plants at 8-day. At any sampling time, most of the absorbed ^{14}C (ca. 91-94 %) was found from the aerial parts. Total recovery of ^{14}C was ca. 98-101 %, which means no occurrence of the loss of ^{14}C by such a phenomenon as evaporation. ARG of the plants (Fig. 4) shows the rapid translocation of absorbed ^{14}C throughout the plants by the clear image of the whole plants. The remarkable darkness at leaf margins (8-day) indicates the acropetal translocation, similarly to the cases of the other treatments.

Fig. 5 shows the results of the fractionation of ^{14}C in the aerial parts and the nutrient solution. The parent compound accounted for 95.3 % of ^{14}C in the nutrient solution (54.7 % of the dose) and metabolic or degradation products were small. NTN 33893 has been proved to be stable in the buffers of pH 4 and pH 7 (half life > 1 year at 20°C)[1]. Thus, the applied ^{14}C-NTN 33893 is considered to be absorbed by eggplants mainly in the unchanged form. Of the absorbed ^{14}C in the aerial parts, 40.3 % (12.9 % of the dose) was the parent compound and the remainder, ca. 60 %, was distributed in

each fraction as metabolites or degradation products as shown in the figure. This suggests that ^{14}C-NTN 33893 was metabolized in eggplants after absorption via roots, but that the activity of the metabolism by plants (without photodegradation on the leaf surfaces) is relatively small, for the proportions of the parent compound and the organo-soluble ^{14}C was greater than those in foliar application. The sum of the residual amount of the parent compound in the plants and the nutrient solution was 67.6 % of the dose.

3. Absorption and translocation of ^{14}C in rice plants

3-1. Leaf blade application and leaf sheath application

After ^{14}C-NTN 33893 was painted on both surfaces of the leaf blades (Table 5), the translocation of ^{14}C to the non-applied parts including the nutrient solution was insignificant and more than 99 % of the retrieved ^{14}C was found from the leaf wash and washed leaves. The dark areas on ARG (Fig. 6) were mainly the applied site and the upper parts, and in the lower parts of the plants, only weak darkness was found at the leaf sheath of the applied leaf at 8-day. In the case of the leaf sheath application (Fig. 6), the translocation of ^{14}C was observed only in the 3rd leaf blade at 1-day, and the accumulation of ^{14}C to the tip of the leaf blade was significant at 8-day. Faint darkness at the lower part of the applied sheath and at the tip of other leaf blades (2nd, 4th and 5th) was also detected at 8-day. This would be explained that the label moved downward from the application site was absorbed to the other sheaths at the bottom of the aerial parts and was translocated acropetally. These results indicates that the translocation of ^{14}C applied to leaf blades and leaf sheaths was mainly acropetal and the basipetal movement was small.

Table 5 shows also that the proportion of ^{14}C in washed leaves increased and the total recovery decreased with the passage of time after application. This and the fact that the content (%) of the parent compound differed between leaf wash and washed leaves (stated below) suggest that ^{14}C-NTN 33893 was photo-degraded on leaf surfaces and penetrated into leaves, and part of the degradation products were volatilized, as found in the case of eggplants.

Fig. 7 shows the fractional distribution of ^{14}C in leaf wash and washed aerial parts at 8-day. In leaf wash, the parent compound amounted to 42.8 % (31.7 % of the dose) and the other label was considered to be composed of conversion products through photodegradation and so forth. Of ^{14}C in washed aerial parts, 13.6 % (3.0 % of the dose) was the parent compound. The difference in % of the parent compound between these two analytical parts was similar to the case of eggplants. It was characteristic in rice plants that the proportion of non-extractable ^{14}C was relatively large as shown in the figure. The total residual amount of the parent compound was 34.7 % of the dose.

3-2. Nutrient solution application

The distribution of ^{14}C applied to the nutrient solution of rice plants is shown in Table 6. ^{14}C was absorbed from the nutrient solution to the plants, though the rate of uptake was smaller than that of eggplants, and 11.3 % of the applied ^{14}C was recovered from the plants at 8-day. The absorbed ^{14}C was distributed predominantly in the aerial parts than roots. The proportion of ^{14}C in the aerial parts increased with the elapse of time, accounting for ca. 94 % at 8-day. The rapid translocation of absorbed ^{14}C was indicated by ARG (Fig. 8). The image of the whole rice plants was clear at 1-day, and the intensive darkness at the leaf tips was significant at 8-day. The acropetal translocation of ^{14}C was remarkable in this treatment as well.

The fractionation of ^{14}C in the aerial parts and the nutrient solution (Fig. 9) shows that 97.0 % of ^{14}C in the nutrient solution (83.7 % of the dose) was the parent compound, indicating the good stability of the applied ^{14}C-NTN 33893 in the nutrient solution of rice plants. Although the absorbed ^{14}C-NTN 33893 was metabolized, 43.8 % (6.3 % of the dose) remained intact. The relatively large amount of the unchanged parent compound and the distribution pattern of ^{14}C in the fractions (Fig 9) indicates that the activity of the conversion of root-absorbed ^{14}C-NTN 33893 in rice plants was smaller than that after leaf surface application. The residual amount of the parent compound in the plants and the nutrient solution at 8-day

was 90.0 % of the dose.

4. Conclusion

The absorption and translocation of ^{14}C-NTN 33893 applied to eggplants and rice plants was similar in both plants as follows.

Part of ^{14}C applied to leaf surface, petiole (eggplants) and leaf sheath (rice plants) penetrated into the plants and was translocated mainly to the upper parts. ^{14}C was distributed restrictively in the leaves and accumulated to leaf margines and leaf tips. NTN 33893 underwent photodegradation on leaf surfaces, which facilitated the penetration and volatilization of ^{14}C. The label was further metabolized in the plants, resulting in small residue of unchanged NTN 33893 at the end of the study.

In the case of stem application (eggplants) also, the penetrated ^{14}C showed acropetal translocation. The rapid distribution of ^{14}C throughout the parts upper than the applied site was observed, and the accumulation to the marginal parts was significant.

After nutrient solution application, ^{14}C was absorbed via roots and distributed rapidly in every plant parts. The significant acropetal translocation was shown. NTN 33893 was metabolized in the plants following absorption in unchanged form, although the metabolic action in the plants was not very intensive. Of the absorbed ^{14}C, considerably large portion still remained as unchanged NTǸ 33893.

REFERENCES

1) [redacted] ORIENTIERENDE VERSUCHE ZUM ABIOTISCHEN ABBAU (14. 11. 1986)

Table 1. Stability and volatility of NTN 33893 (Preliminary test)

	Conditions	Results
STABILITY		
Organic solvent	light[a], r.t.[b]	more polar products were formed in all solvents tested[c] (max. 3% of applied ^{14}C, in 34 days)
	dark, r.t.	stable
	dark, 4°C	stable
Thin layer	light[a]	more polar and less polar products were formed (ca. 5% of applied ^{14}C in 11 days)
	dark	stable
Acid/Alkaline	acid (100°C, 2hr)	almost stable, only trace amount of more polar products were formed in 3N and 6N solution
	alkaline (100°C, 2hr)	transformed completely to more polar products (1N NaOH)
VOLATILITY		
Rotary evaporator		non-volatile in all solvents tested[d] (^{14}C in the trap: <0.2%)
Lyophilizer	thin film	non-volatile (^{14}C in the trap: <0.3%)
	covered with 5% CMC	non-volatile (^{14}C in the trap: <0.3%)

a) fluorescent lamp in laboratory
b) room temperature
c) methanol, ethanol, acetone, acetonitrile, dichloromethane, ethyl acetate and toluene
d) methanol, 80% methanol, dichloromethane, acetone and ethyl acetate

Table 2. Time-course distribution of ^{14}C in eggplants and nutrient solution after foliar application of ^{14}C-NTN 33893 via upper surface

Sample	% of total ^{14}C applied[a]			
	1d	2d	4d	8d
Treated leaves	95.6	99.7	89.1	85.6
Leaf wash[b]	67.2	83.9	34.8	30.2
Washed leaves	28.4	15.8	54.3	55.4
Other aerial parts	0.1	0.7	1.0	1.4
Roots	<0.1	<0.1	<0.1	<0.1
Nutrient solution	0.2	0.2	<0.1	<0.1
Total recovery	95.9	100.6	90.1	87.0

a) The values are means for two plants
b) washed by ethanol

Table 3. Time-course distribution of ^{14}C in eggplants and nutrient solution after foliar application of ^{14}C-NTN 33893 via lower surface

Sample	% of total ^{14}C applied[a)]			
	1d	2d	4d	8d
Treated leaves	95.2	97.7	96.8	97.3
Leaf wash[b)]	60.0	72.7	63.0	66.1
Washed leaves	35.2	25.0	33.8	31.2
Other aerial parts	0.2	0.6	0.4	0.4
Roots	<0.1	<0.1	<0.1	0.1
Nutrient solution	0.2	0.7	0.1	<0.1
Total recovery	95.6	99.0	97.3	97.8

a) The values are means for two plants
b) washed by ethanol

Table 4. Time-course distribution of ^{14}C in eggplants and nutrient solution after root application of ^{14}C-NTN 33893 via nutrient solution

Sample	% of total ^{14}C applied[a)]			
	1d	2d	4d	8d
Plant	5.7	11.0	21.8	39.0
Aerial parts	5.2	10.2	20.6	36.8
Roots	0.5	0.8	1.2	2.2
Nutrient solution	91.8	89.4	76.1	62.0
Total recovery	97.5	100.4	97.9	101.0

a) The values are means for two plants.
b) Total water uptake in 8 days: 248 ml/plant

Table 5. Time-course distribution of ^{14}C in rice plants and nutrient solution after foliar application of ^{14}C-NTN 33893

Sample	% of total ^{14}C applied[a]			
	1d	2d	4d	8d
Treated leaves	100.8	99.1	97.2	92.9
Leaf wash[b]	93.6	89.3	83.4	69.0
Washed leaves	7.2	9.8	13.8	23.9
Other aerial parts	<0.1	0.1	0.2	0.5
Roots	<0.1	<0.1	<0.1	<0.1
Nutrient solution	<0.1	<0.1	<0.1	0.1
Total recovery	100.8	99.2	97.4	93.5

a) The values are means for two plants
b) washed by ethanol

Table 6. Time-course distribution of ^{14}C in rice plants and nutrient solution after root application of ^{14}C-NTN 33893 via nutrient solution

Sample	% of total ^{14}C applied[a)]			
	1d	2d	4d	8d
Plant	0.9	1.7	3.5	11.3
Aerial parts	0.7	1.5	3.1	10.6
Roots	0.2	0.2	0.4	0.7
Nutrient solution	96.9	97.0	94.3	80.1
Total recovery	97.8	98.7	97.8	91.4

a) The values are means for two plants.
b) Total water uptake in 8 days: 61 ml/plant

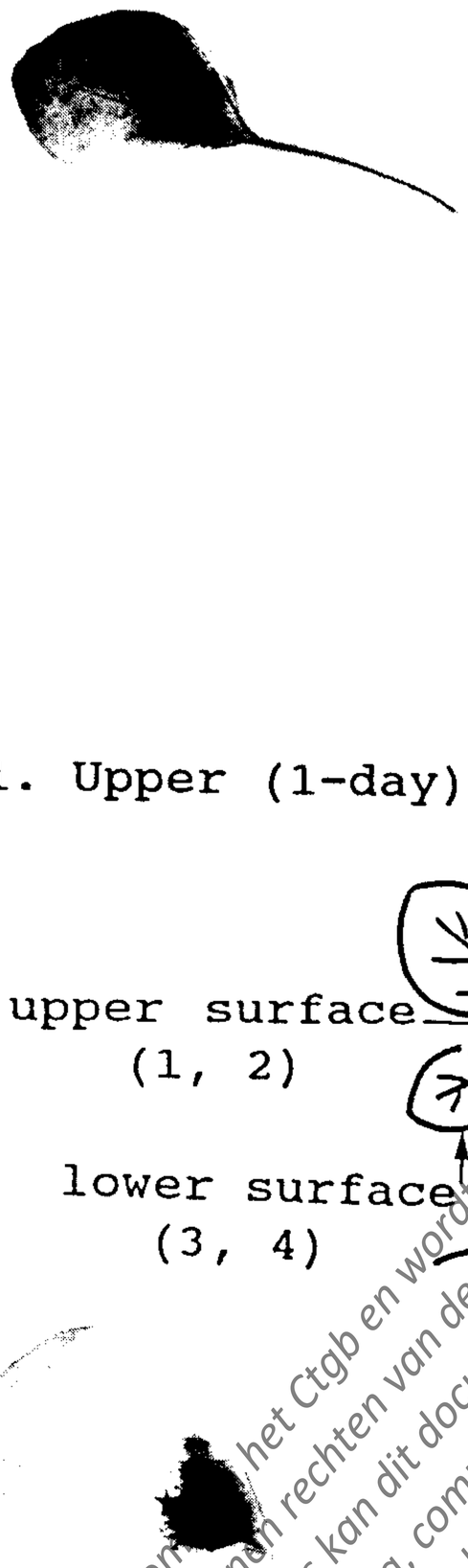


Fig. 1 Autoradiographs of eggplants 1 day and 8 days after foliar application of ^{14}C-NTN 33893 via upper surface (1, 2) and lower surface (3, 4).

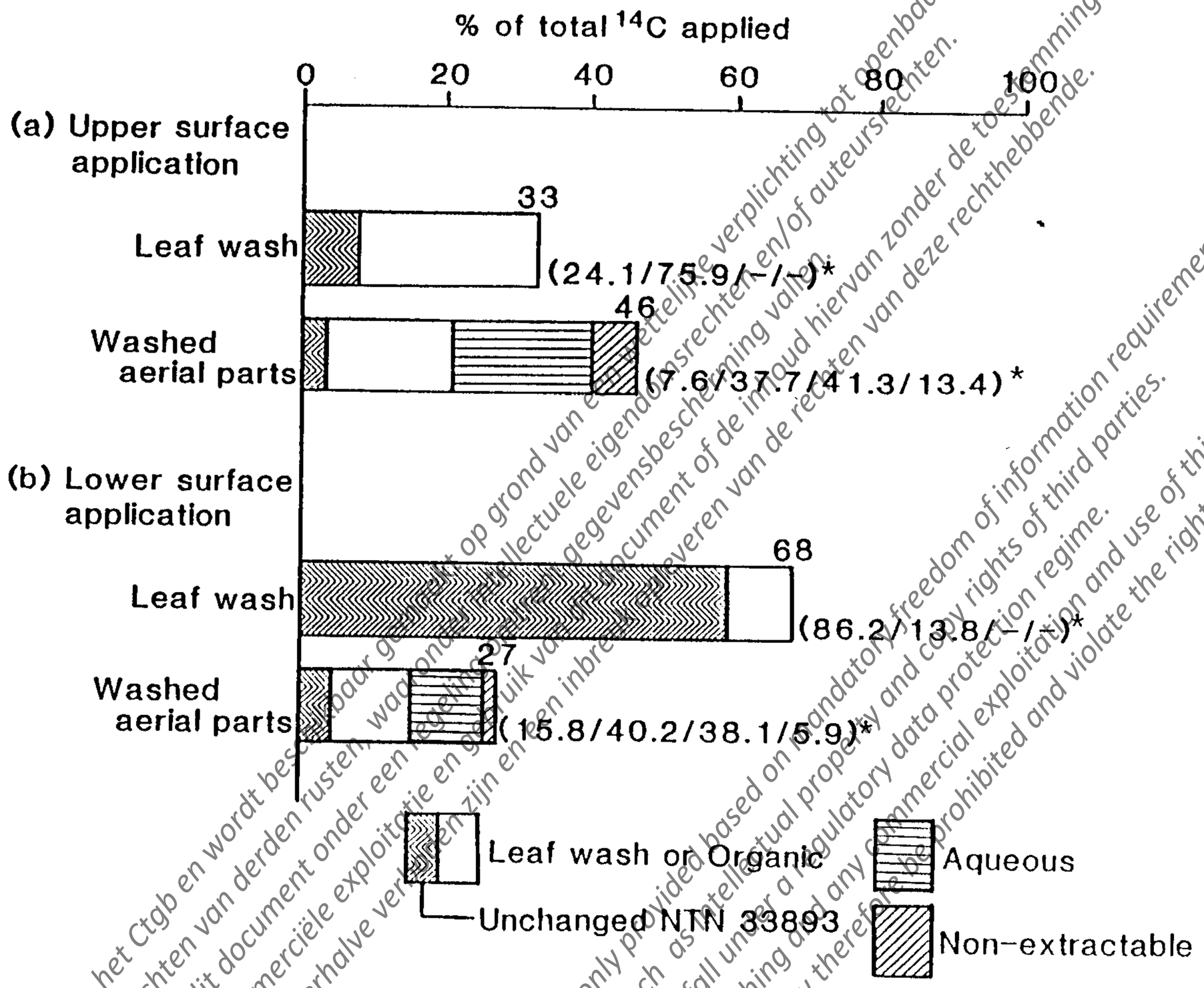


Fig. 2 Fractional distribution of ^{14}C in leaf wash and washed aerial parts 8 days after foliar application of ^{14}C-NTN 33893 to eggplants.

* proportion of NTN 33893/Org.(except NTN 33893)/Aqu./Non-ext. in % of ^{14}C found from each analytical part

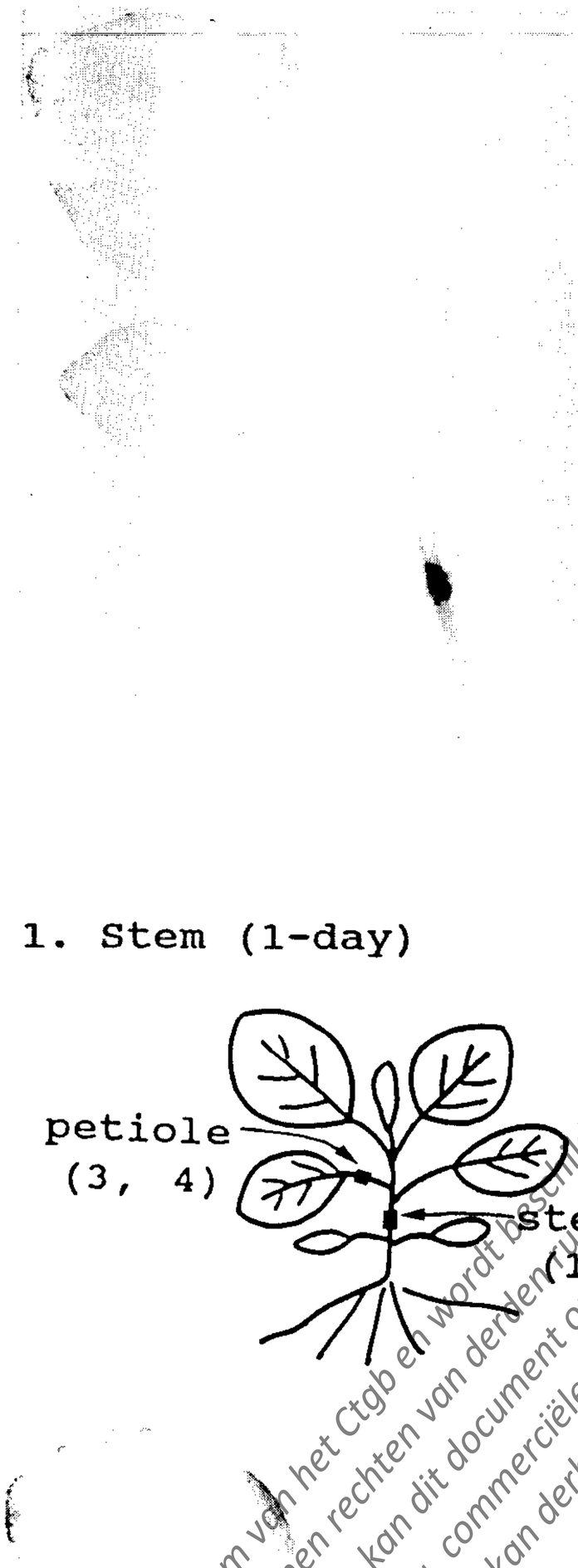


Fig. 3 Autoradiographs of eggplants 1 day and 8 days after stem (1, 2) and petiole (3, 4) application of ^{14}C-NTN 33893.

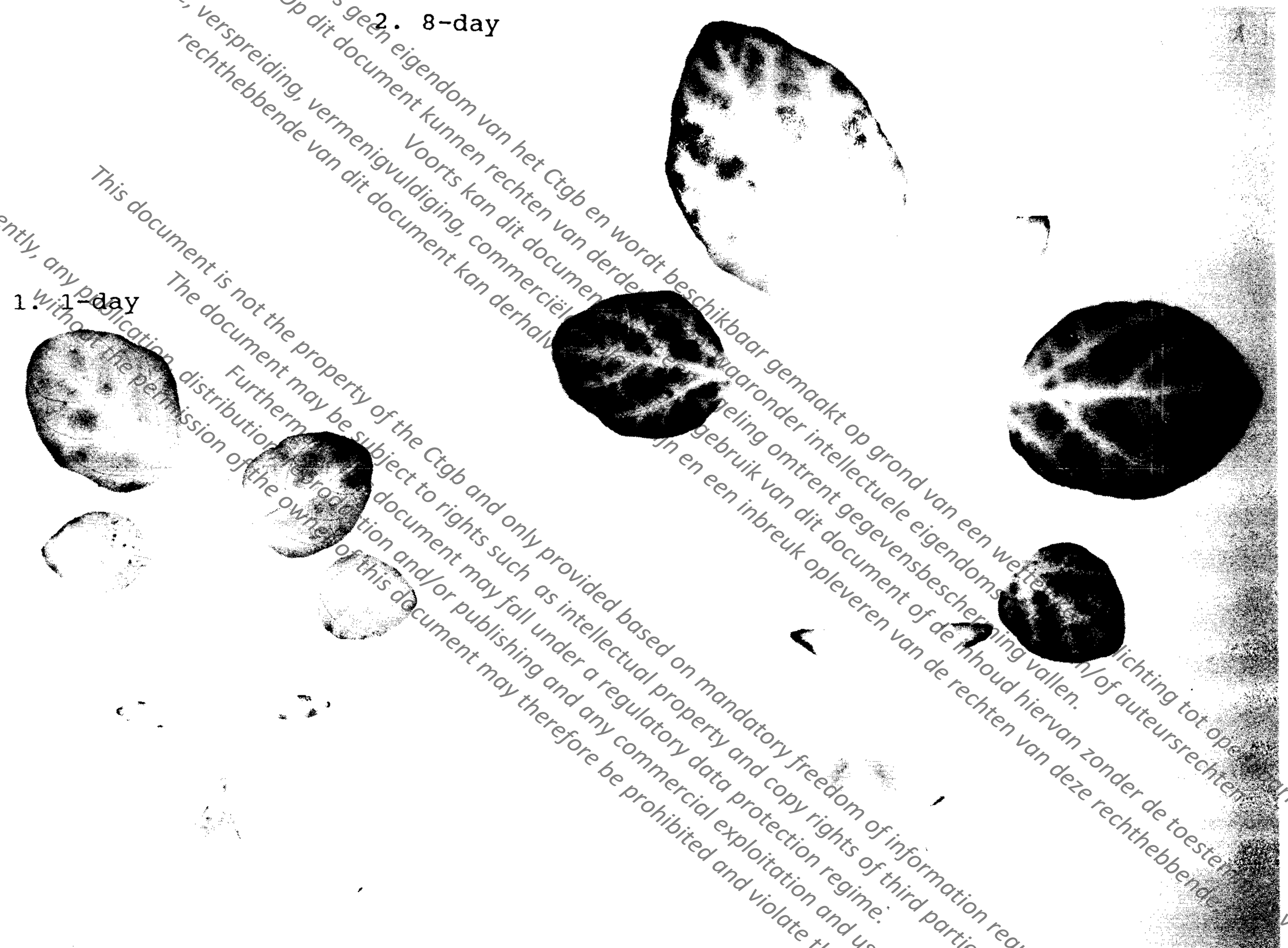


Fig. 4 Autoradiographs of eggplants cultivated in ^{14}C-NTN 33893-treated nutrient solution for 1 day (1) and 8 days (2).

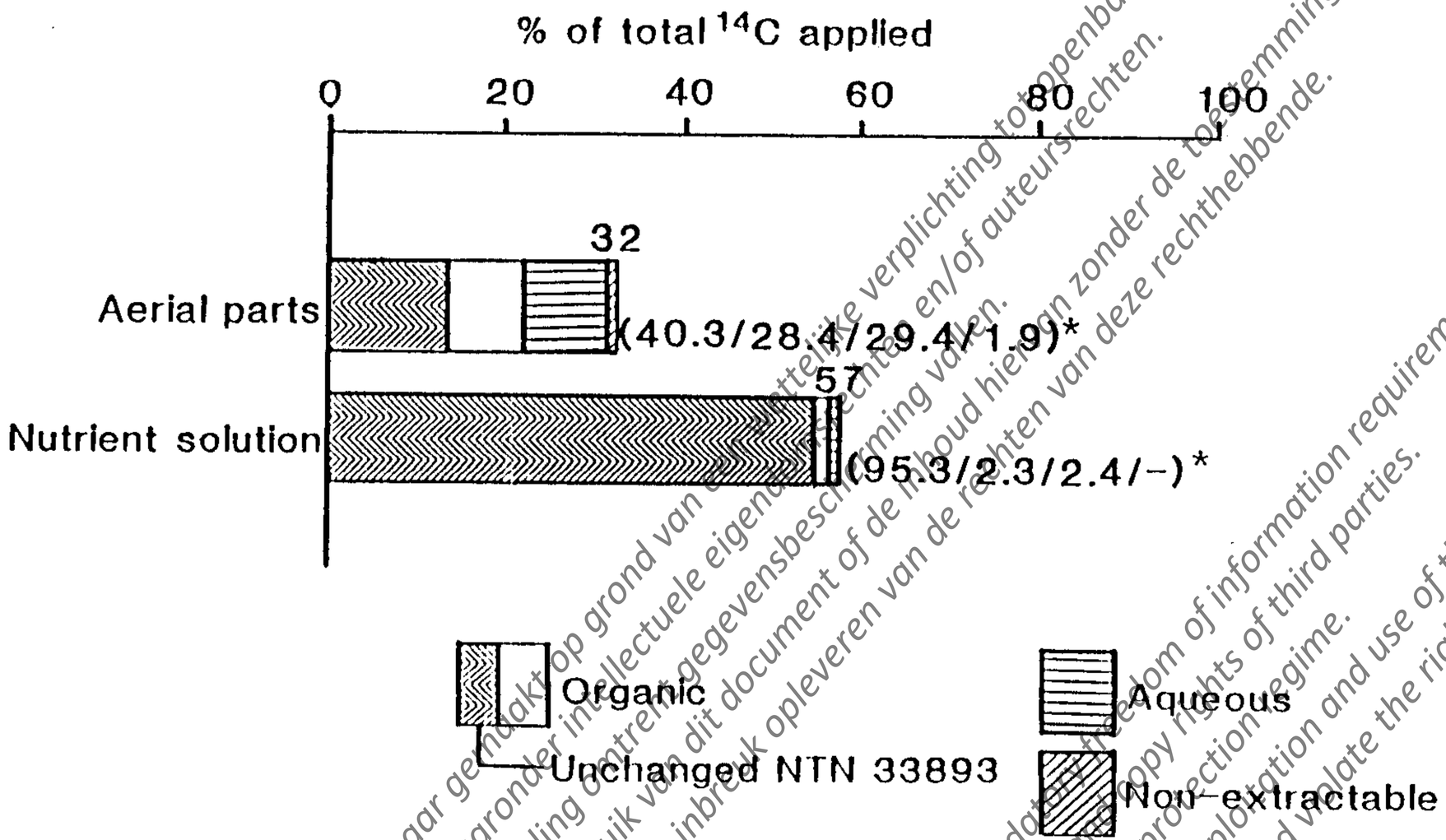
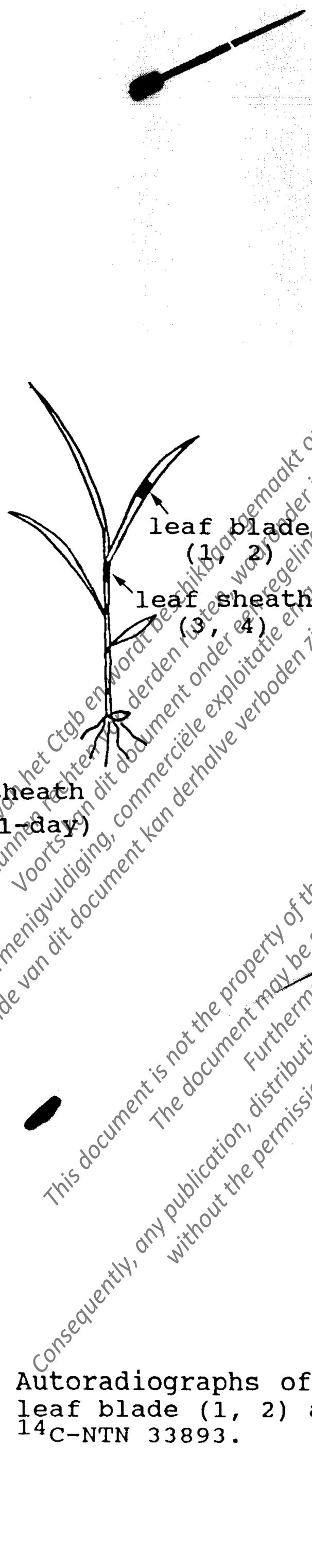


Fig. 5 Fractional distribution of ^{14}C in aerial parts and nutrient solution 8 days after cultivation of eggplants in ^{14}C-NTN 33893-treated nutrient solution.

* proportion of NTN 33893/Org.(except NTN 33893)/Aqu./Non-ext. in % of ^{14}C found from each analytical part

1. Leaf blade (1-day)

2. Leaf blade (8-day)



3. Leaf sheath
(1-day)

4. Leaf sheath
(8-day)

Fig. 6 Autoradiographs of rice plants 1 day and 8 days after leaf blade (1, 2) and leaf sheath (3, 4) application of ^{14}C-NTN 33893.

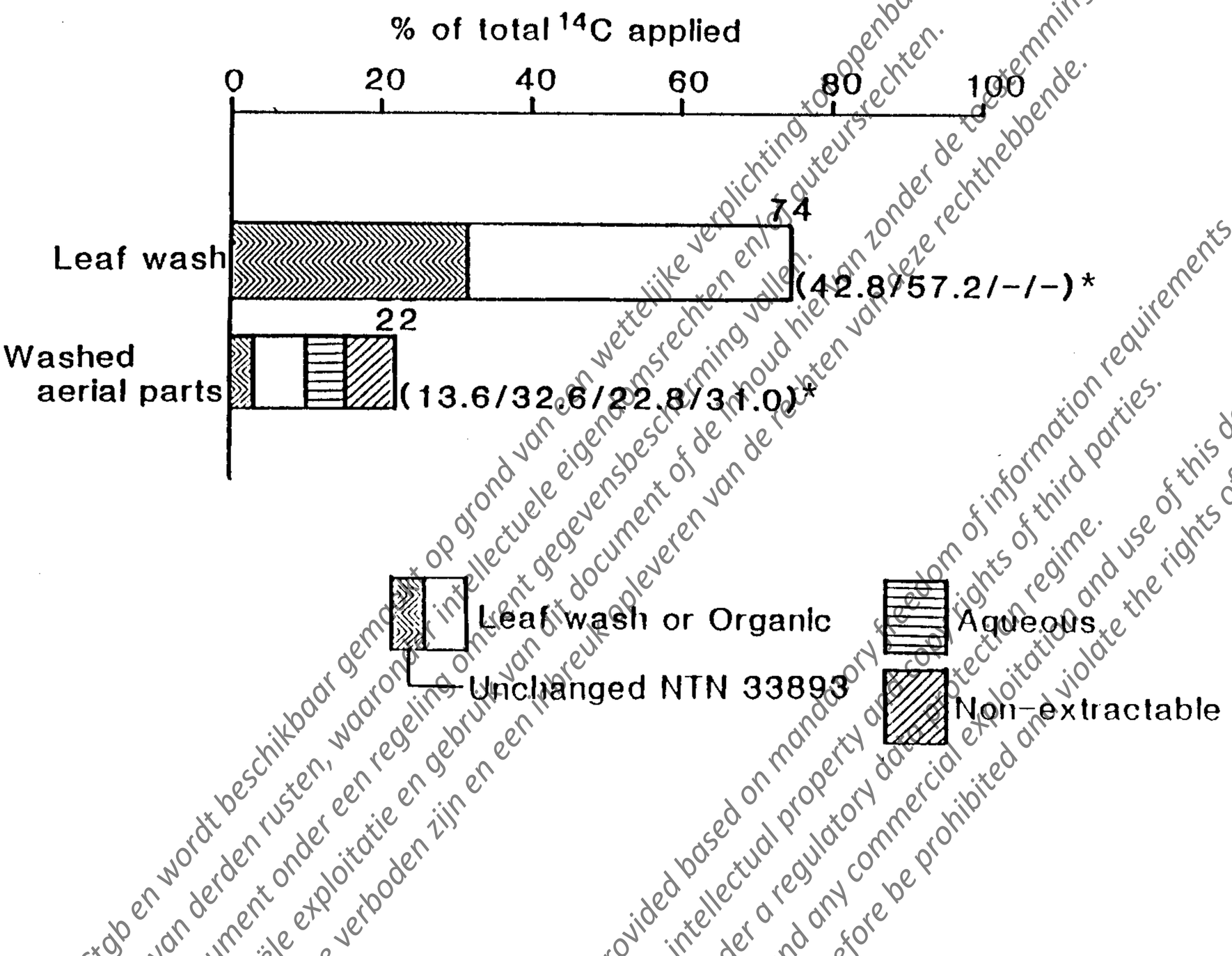


Fig. 7 Fractional distribution of ^{14}C in leaf wash and washed aerial parts 8 days after leaf blade application of ^{14}C-NTN 33893 to rice plants.

* proportion of NTN 33893/Org.(except NTN 33893)/Aqu./Non-ext. in % of ^{14}C found from each analytical part

1. 1-day

2. 8-day

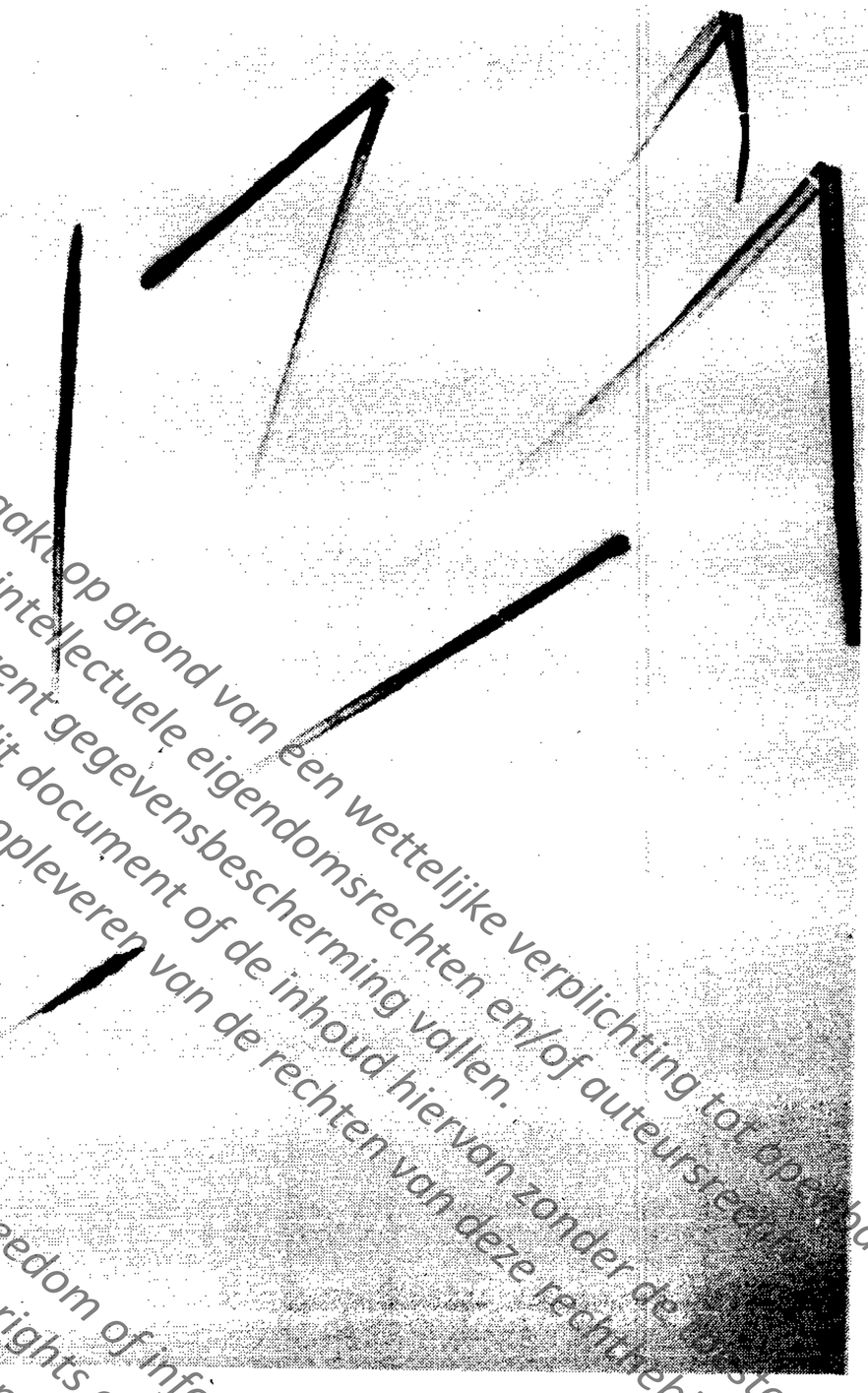


Fig. 8 Autoradiographs of rice plants cultivated in ^{14}C-NTN 33893-treated nutrient solution for 1 day (1) and 8 days (2).

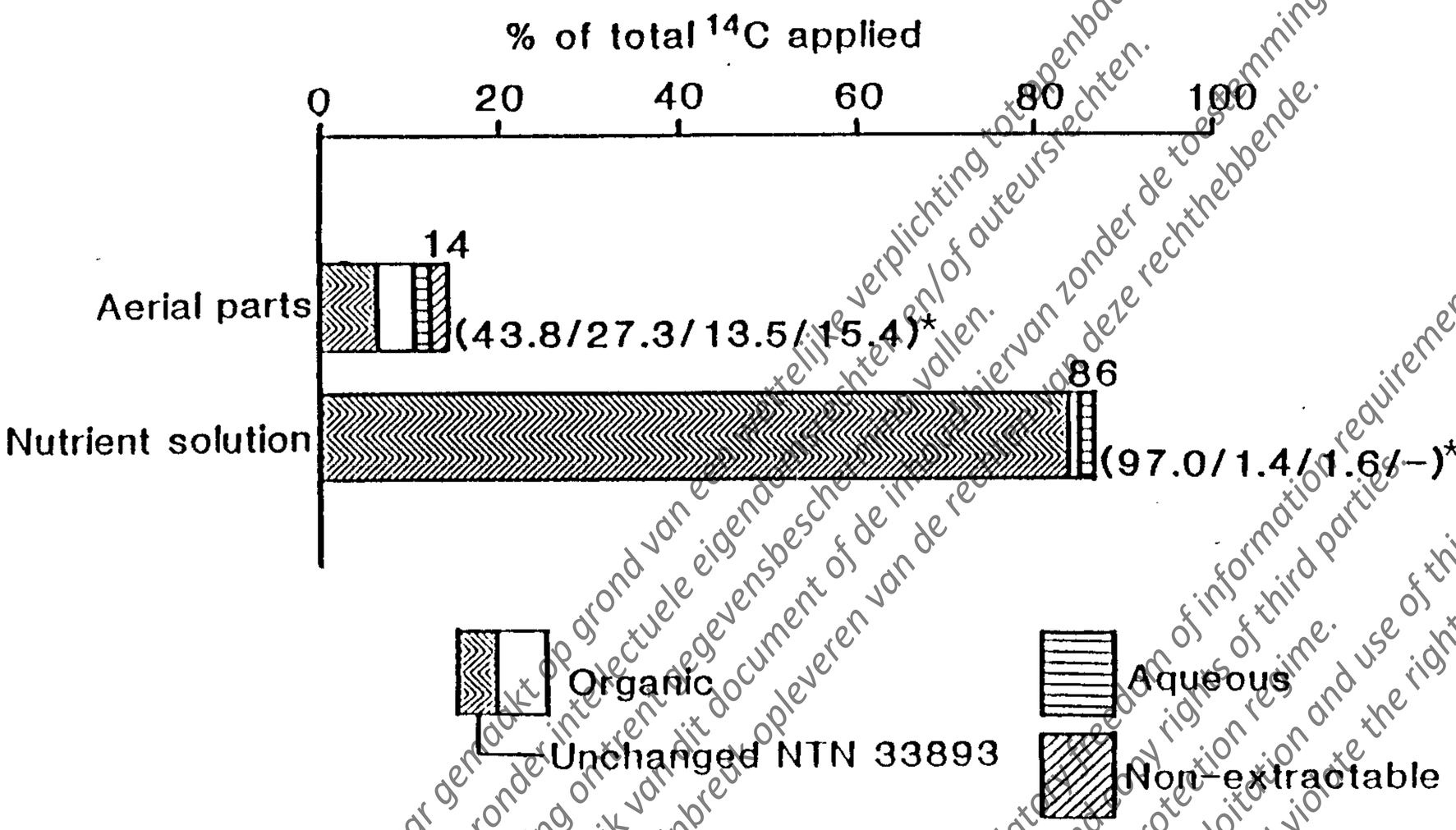


Fig. 9 Fractional distribution of ^{14}C in aerial parts and nutrient solution 8 days after cultivation of rice plants in ^{14}C-NTN 33893-treated nutrient solution.

* proportion of NTN 33893/Org.(except NTN 33893)/Aqu./Non-ext. in % of ^{14}C found from each analytical part