The Kinetics and Energy of Activation of the Esterification of Acetic Acid and Ethanol in the Presence of Sulfuric Acid. Gary E. Carnahan, David Lipscomb College. The Fischer esterification reaction has long been held important by physical organic chemists—since its use by Guldberg and Waage in 1897 to develop the law of mass action. A study was made of the reaction of glacial acetic acid and absolute ethanol in the presence of sulfuric acid which produces ethyl acetate and water. This reaction was shown to follow second order kinetics. The technique used to follow the progress of the reaction was to determine the decreasing area of the quartet due to the methylene protons of ethanol in the nmr spectrum at varying times. The application of the Arrhenium equation to the second order rate constants and the temperatures at which these were detremined allowd the calculation of the energy of activation for the esterification as 7.22 kcal/mole.

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THE RELATIONSHIP OF EARTHWORM (*OLIGOCHAETA: ACANTHODRILIDAE* AND *LUMBRICIDAE*) DISTRIBUTION AND BIOMASS IN SIX HETEROGENEOUS WOODLOT SITES IN TIPPECANOE COUNTY, INDIANA.

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ABSTRACT

Observations on earthworm populations have been made in Tippecanoe County, Indiana to determine the distribution, species complex, population density and biomass estimates in six woodlots under different ecological conditions. The survey shows that six species are present, representing five genera. All six species have been previously recorded in other areas of Indiana.

The maximum number of species occurring in any single woodlot population was four, two being the more usual. In 1968 there was *Lumbricus terrestris* in combinations with *Bimastos zeteki, Dendrobaena octaedra,* and *Octolasion tyrtaeum.*

The relative abundance and biomass of earthworms appear to decrease with a decrease in soil moisture, soil acidity, and palatability of the food source. The elm and maple species being the more palatable while oak and beech species the less palatable.

The equations determined for dry weight and wet weight to length of earthworms are: $\log D = 0.4055 + 2.3900 \log L$ and $\log W = 0.2800 + 2.3362 \log L$ where L = earthworm length in cm, D = dry weight in mg, and W = wet weight in mg. The correlation coefficient for both of these equations is 0.91.

Earthworm densities ranged from a minimum of 14.2 to 142.4 per m^2. The range of wet weight biomass estimates were 26.29 to 280.28 g/m^2. The maximum biomass value was > 100% of the values reported in American and European literature for comparable ecosystems.

INTRODUCTION

This study was undertaken to determine the earthworms present in six heterogeneous woodlot sites during the summers of 1967 and 1968. These sites have been utilized by other researchers in the Entomology Department at Purdue University and yielded considerable ecological data. This study is the first attempt to evaluate oligochaete information from these woodlots.

For more than a half century, distribution records for Indiana have been published. In 1914, Heimburger published the first regional paper on the earthworm fauna of Indiana. His preliminary list of 14 species included *Bimastos (Helodrilus) zeteki* Smith and Gittins 1915, *Diplocardia singularis* Ude, 1893, and *Octolasium lacteum* (Örley, 1881) which is a synonym for *Octolasion tyrtaeum* (Savigny, 1826) (Gates, personal communication). This error has gone unchecked for decades resulting in frequent *Octolasium lacteum* citations incorrectly appearing in the literature. Smith (1917) added the 15th species. Gates (1942) increased the state list to 16 species. Then Chandler (1953) added ten species to bring the total to 26, including *Dendrobaena octaedra* (Savigny, 1826), *Lumbricus rubellus* Hoffmeister, 1843, and *Lumbricus terrestris* L. Finally Joyner (1959) listed two additional species to the state list and verified four of Chandler's records, including *D. octaedra, L. rubellus,* and *L. terrestris.*

Edwards (1967) found that the expression of soil animal populations as biomass is more meaningful than numbers; this is particularly true when considering the productivity of a habitat. Biomass values reported in the literature are confusing because rarely, if ever, is it stated whether they are wet or dry weights. To illustrate, Bornebusch (1930) stated "the earthworms collected were sorted according to species, counted, and weighed." After reviewing the original European papers, I assumed that Bornebusch's values and those summarized by Satchell (1967) are wet weights.

Water ordinarily constitutes 80-90% of the living earthworm's weight (Grant, 1955), although earthworms possess a remarkable ability to withstand desiccation of 70% or more of their water content (Roots, 1956). Therefore, only dry weight biomass estimates should be used in comparing the productivity of earthworm populations from different habitats.

SITE CHARACTERISTICS

The sampling sites were selected to provide a number of clear-cut seres in a deciduous forest succession and several physiographic soil types. The soil pH values were 1:1 KCl determinations. The vegetative classes were: seedlings—less than 2.5 cm dbh, saplings—2.5 to 10.2 cm dbh, and trees—greater than 10.2 cm dbh. The sites were 0.08 hectare circles of 15 meter radius.

Wabash:

This site was on the flood plain of the Wabash River bordering the Purdue University Veterinary Farm. The soil of this moderately well drained bottomland is an Eel silty clay loam, 0 to 3% slope. These soils were developed from neutral to slightly calcareous alluvium from timbered glacial drift. The Eel series is a member of a fine-loamy, mixed, mesic family of Aquic Fluventic Eutrochrepts. The pH of the upper profile was 6.7 with an 02 horizon of approximately 0.6 cm. The dominant tree species are silver maple (*Acer saccharinum L.*) and Cottonwood (*Populus deltoides Bartr.*). In approximately a 2:1 ratio

(69.8:27.9%) but only silver maple is reproducing. Silver maple comprises 73.6% and 89.1% of the sapling and seedling classes respectively.

Open Oak:

A grazed oak stand located south-west of the Purdue University Airport, now partially cleared for runway extension in 1969, was another site. The soil of this normally well drained terrace is a Fox loam, 0 to 3% slope. Periodic grazing has had a compaction effect on the upper portion of the profile. These soils were derived from loam over calcareous sand and gravel. The Fox series is a member of a fine-loamy, mixed, mesic family of Typic Hapludalfs. The pH of the upper profile was 4.9 with 01 and 02 horizons of 1.25 cm each. The tree class is 100% white oak (*Quercus alba* L.), with no sapling class. The seedling class is composed of four main species: apple (*Malus*), black currant (*Ribes nigrum* L.), hackberry (*Celtis occidentalis* L.) and red elm (*Ulmus rubra* Muhl.) comprising over 85% of this class.

Black Locust:

A third site was located in a black locust stand on the Purdue University Horticulture Farm. The soil of this well drained upland glacial till is a Miami silt loam, 0 to 3% slope. The Miami series is a member of a fine-loamy, mixed, mesic family of Typic Hapludalfs. The pH of the upper profile was 4.2 with an 01 horizon of 2.5 cm. By far the majority (82%) of woody plants in the tree size are black locusts (*Robinia pseudoacacia* L.) with red elm comprising 15.6%. The black locusts are reproducing only along the margins of the stand, with the interior sapling and seedling classes being largely red elm (87.7 and 52.1%). In addition, seedlings of white ash (*Fraxinus americana* L.), *black walnut* (*Juglans nigra* L.) and sugar maple (*Acer saccharum* Marsh.) show signs of soon becoming prominent.

Stewart Beech:

A beech-maple stand located on the Stewart estate on Airport Road near West Lafayette was the fourth site. The soil is another well drained Miami silt loam, 0 to 3% slope. The pH of the upper profile was 5.5 with 3 to 5 cm of 02 horizon. There is no dominant species in the tree class: Basswood (*Tilia americana* L.) 26.3%, beech (*Fagus grandifolia* Ehrh.) 17.9%, slippery elm (*Ulmus fulva* Michx.) 19.3% and sugar maple 12.3%. In the seedling class there is a great number of species present with sugar maple (73.8%) decidedly dominant followed by white ash (9.1%). Beech is not reproducing well, averaging only 1% in the sapling and seedling classes. Blue beech (*Carpinus caroliniana* Walt.), hop hornbeam (*Ostrya virginiana* Koch.) and sugar maple are important as understory species with 38.8%, 18.8% and 10.1% of the saplings.

McCormick Woods:

Yet another site was a mixed stand on the northern portion of McCormick's Woods. The soil of this poorly drained upland calcareous glacial till is a Brookston silty clay loam, 0 to 3% slope. The Brookston series is a member of a fine-loamy, mixed, mesic family of Typic Argiaqualfs. The pH of the upper profile was 5.8 with about 1.8 cm of 01 horizon. In the tree class black ash (*Fraxinus nigra* Marsh.), white ash and silver maple are the dominant species with 25.0%, 18.7% and 12.5% of the trees respectively. Of these only white ash is reproducing with 8.7% of the seedlings and 6.4% of the saplings. The sapling and seedling classes are dominated by spice bush (*Lindera Benzoin* (L.) Blume) which represents 40.0 and 57.7 percent of these classes. Red elm is second in these classes with 20.0 and 13.7 percent respectively.

Bremmer:

A mixed stand located on State Highway 26 approximately one mile east of County Road 550 constitutes the final site. The soil of this imperfectly drained upland glacial till is a Fincastle silt loam, 0 to 3% slope. This soil is one of the most extensive soils in Tippecanoe County (USDA, 1959). The Fincastle series is a member of a fine-loamy, mixed, mesic family of Aeric (Typic) Ochraqualfs. The pH in the upper profile was 4.6 with an 02 horizon of 5.1 cm. The site is going from a white oak - sugar maple stand to predominantly sugar maple stand. White oak, sugar maple and red elm make up 36.0, 28.8 and 12.0 percent of the tree class. Sugar maple comprises 42.7% and 64.4% of the sapling and seedling classes, respectively, while red elm represents 18.3% and 14.1% of these classes.

Methods

There are numerous methods for sampling earthworm populations. The effectiveness of these methods varies depending upon the species and habitat; no one method is equally suitable for all species and all habitats. The most reliable sampling method is digging and hand-sorting (Satchell, 1955; 1967; 1969). This study, however, utilized the expellant of earthworms from the soil by the formaldehyde solution (Raw, 1959) since digging and handsorting would destroy these habitats for future experiments.

On each of these sites (Table 1) ten 0.25 m^2 (1967) and five 0.25 m^2 (1968) areas of soil surface were sampled. A solution of 25 ml of formalin and four liters of water was sprinkled over each quadrat. The earthworms that surfaced in ten minutes following the application of the expellant were collected. Sampling was conducted during August 1967 and August 1968.

Biomass values were determined by measuring the length of 25 dead gut voided earthworms. These worms were then placed in a tared aluminum dish and oven-dried at 105° C for 24 hours. After approximately 20 minutes in a desiccator, the worms and dishes reached room temperature and were quickly weighed. Because of the wet weight - dry weight discrepancy in the literature, a conversion factor was required before the data from this study could be compared with the live weights of other reports. Fourteen live earthworms were collected, gut voided, weighed live, killed, measured, and then oven-dried and reweighed as previously described. Equations for the relationship between length and weights are found in the results.

Results and Discussion

The results of the earthworm collections are presented in Tables 1 and 2. The first table illustrates the earthworm distribution by species and site for the two sampling periods. The second table presents the total density and biomass for each site in the same periods.

TABLE I. Earthworm distribution on six woodlot sites during sampling periods in 1967 and 1968 in Tippecanoe County, Indiana.

Site	Species	1967[1,2]	1968[1]
Wabash	*Bimastos zeteki*		70- 8-17
	Dendrobaena octaedra	3- - 0	
	Lumbricus rubellus	4- - 7	
	Lumbricus terrestris	15- - 4	35- 3- 1
	Octolasion tyrtaeum	2- - 0	
Open Oak	*Lumbricus terrestris*		2- 0- 0
	Octolasion tyrtaeum	17- -20	43- 8-39
Black Locust	*Bimastos zeteki*		1- 0- 0
	Dendrobaena octaedra	121- -99	12- 0- 0
	Lumbricus rubellus	1- - 1	
	Lumbricus terrestris	1- - 1	3- 2- 0
	Octolasion tyrtaeum	4- -14	0- 1- 0
Stewart Beech	*Bimastos zeteki*		41-25- 9
	Dendrobaena octaedra		1- 0- 3
	Lumbricus terrestris	26- - 1	61-15-22
	Octolasion tyrtaeum	2- - 0	
McCormick	*Bimastos zeteki*		88-11- 1
	Dendrobaena octaedra	1- - 0	
	Lumbricus terrestris	1- - 0	52- 8-12
Bremmer	*Diplocardia singularis*		3- 1- 1
	Lumbricus terrestris		3- 0- 0
	Octolasion tyrtaeum	96- -29	59-12-37

[1]Trinomials after species indicate: juveniles - aclitellate adults - clitellate adults.

[2]Data for 1967 did not distinguish between aclitellate and clitellate adults.

The precipitation during the spring and summer of 1967 was less than normal for that period of the year, but the spring and summer of 1968 were much wetter than normal (Table 3). For example, there were 23 consecutive days of rain in May of 1968. The surface soil was noticeably wetter the second year during the sampling period. Also, the surface vegetation was noticeably more luxurious in 1968 compared to the previous year. In all sites, except Black Locust, there was a significant increase in earthworm density from 1967 to 1968. Thus earthworm densities increased with increases in soil moisture. This pattern has been reported previously. (Evans and Guild, 1947; Satchell, 1967).

TABLE II. Earthworm densities and biomass values for six woodlot sites sampled in 1967 and 1968 in Tippecanoe County, Indiana.

Site	1967 No./m^2	1968 $\bar{x}$ 0.25 m^2 (± S. E.)	1968 No./m^2	1968 Dry Wt. g/m^2	1968 Wet Wt. g/m^2
Wabash	14.00	28.80(4.05)	115.20	40.44	222.42
Open Oak	14.80	18.40(4.92)	73.60	26.68	146.74
Black Locust	96.80	3.80(1.68)	14.20	4.78	26.29
Stewart Beech	13.80	35.60(4.37)	142.40	13.84	76.12
McCormick	0.80	34.40(6.37)	137.60	50.96	280.28
Bremmer	50.00	23.20(2.44)	92.80	33.47	184.08

Diplocardia singularis (Acanthodrilidae) was the only species in this study which is not in the family Lumbricidae. This species was collected in small numbers from only one site (Bremmer). Members of this genus are strictly nearctic (Eisen, 1899). They are found in both cultivated and relatively undisturbed soils in wooded areas (Causey, 1952; Chandler, 1953).

TABLE III. Precipitation data (mm) for West Lafayette (Tippecanoe County), Indiana.[1]

	1967	1968	1953-1963 Average	1901-1963 Average
January	56.64	45.72	41.66	60.45
February	32.51	31.24	45.21	50.00
March	54.61	34.54	59.94	76.71
April	87.12	82.30	96.01	94.23
May	97.79	210.57	104.65	104.65
June	27.18	147.32	110.49	102.11
July	29.46	91.69	137.41	99.57
August	67.31	116.08	91.95	86.11
September	36.07	28.96	59.44	84.07
October	108.46	29.97	73.41	74.42
November	61.47	119.63	62.23	65.53
December	172.97	52.32	36.32	57.15
Total	831.60	972.57	918.72	955.04

[1]Data supplied by L. A. Schaal, State Climatologist, Purdue University, Agronomy Department, Lafayette, Indiana.

Members of the genus *Bimastos* are primarily nearctic, with a high percentage of endemic species, which are found in and around decayed leaf mold and logs. The three remaining lumbricids *Dendrobaena, Lumbricus* and *Octolasion* are introduced European genera. These three are often found in wooded areas and around leaf mold as well (Causey, 1952; Chandler, 1953). Guild (1951) found that earthworm species are always found in associations, usually seven or eight species, with a maximum of ten and a minimum of four species. Both Guild (1951) and Chandler (1953) found *Lumbricus rubellus, L. terrestris,* and *Octolasion tyrtaeum* together on the same site. Reynolds (1969 and 1970) found many associations in two distinct regions of east Tennessee. At Oak Ridge in 1969, I found *Diplocardia* and *Octolasion tyrtaeum* occurred together exclusively 100% of the time. In the same year, on other sites at Oak Ridge, Reynolds (1970) found *Diplocardia* alone and in combinations with *Bimastos* or *Octolasion*. In this same study, I found *Dendrobaena octaedra* with *Octolasion tyrtaeum* 50% of the time but never with *Diplocardia.*

In the Great Smoky Mountains National Park, I found *Octolasion tyrtaeum* in all sites sampled (Reynolds, 1969). At the lower elevations (below 2000 feet), *Octolasion* was found with *Bimastos* and *Diplocardia.* Between the 2000 and 3000 foot elevations, these latter two genera were replaced by *Dendrobaena octaedra* and in one site by *Lumbricus rubellus.*

The results presented in Table 1 show the associations found in this study. *Bimastos zeteki* and *Diplocardia singularis* were not collected the first year and *Lumbricus rubellus* was absent the second year. Also, *Dendrobaena octaedra* was generally reduced in numbers the second year. *Lumbricus terrestris* was present in all sites the second year. The species associates in 1968 were: *Bimastos zeteki - Lumbricus terrestris* at Wabash, Stewart Beech and McCormick, *Dendrobaena octaedra - Lumbricus terrestris* at Black Locust, while *Octolasion tyraeum - Lumbricus terrestris* were at Open Oak and Bremmer. The only significant differences the previous year were the absence of *Bimastos zeteki* in all sites and the *Dendrobaena octaedra - Octolasion tyrtaeum* association at Black Locust.

Studies in leaf litter breakdown have been conducted by Edwards and Heath (1963) and Heath *et al.* (1966). They found the following disappearance rates of leaf discs in small mesh bags in woodland soil to be: elm > (ash = lime (*Tilia*)) > oak = beech. When a large mesh was used to allow earthworms to act on the leaf discs the same order of disappearance was observed. Sometimes the rates of oak and beech disappearance were close but oak always disappeared before beech. Their studies did not include maple. In 1968 under the direction of Edwards, Reynolds and McBrayer (unpublished data) compared the action of earthworms (*L. terrestris*) on the disappearance of elm and maple. We found that after the first few weeks the disappearance rates of these two species were almost identical. These studies above corroborated the work and observations of earlier workers that leaf disappearance and palatability for earthworms are positively correlated, i.e. the quickly disappearing species (elm and maple) are more palatable than the slowly decomposing species (oak and particularly beech).

These factors seem to be reflected by the data of this study. If the sites are ranked in decreasing biomass values for earthworms, the list is: McCormick, Wabash, Bremmer, Open Oak, Stewart Beech, and Black Locust. McCormick's Woods has a maple-ash tree class and elms dominate in the seedling class. The second site (Wabash) is a maple-basswood site. Bremmer is the third site, although oaks are the dominant species in the tree class, they are not reproducing while maple and elm are replacing oak in large numbers in the sapling and seedling classes. Open oak is a similar situation where palatable species are taking over the smaller size classes. Even though there is no dominant species in Stewart Beech, the effect of the beech has probably caused this site to be fifth in biomass. The 82% black locusts in the tree class has had a profound effect on this last site. This species was not included in the disappearance tests previously mentioned. Black locust litter decomposes very rapidly releasing its soluble nutrients (USDA, 1965). This rapid loss with the increased precipitation on the well drained upland site may have removed the nutrient sources by leaching before they could be utilized by the earthworms.

Although not as clear cut an example, the soil acidity follows a similar trend with the sites having the greatest biomasses being more neutral while the sites with the lowest biomass values have more acidic soil conditions.

Many ecological factors can affect earthworm population densities and the species complex. Undoubtedly, the obvious and most important are soil moisture, soil temperature and soil acidity, each of which can act as a complete limiting factor at extremes. Other factors such as soil depth and texture, nutrient and food supply (vegetation species) and physiographic features can interact with these factors and influence the soil conditions (Guild, 1952).

In this study soil moisture, food source (leaf species) and soil acidity appear to be the more important limiting factors.

The equations for dry weight and wet weight to length of earthworms are:

$$\log D = 0.4055 + 2.3900 \log L \quad \text{and,}$$
$$\log W = 0.2800 + 2.3362 \log L$$

where L = earthworm length in cm, D = dry weight in mg, and W = wet weight in mg. The equation for converting dry weight to weight weight for an earthworm of any given length is:

$$\log W = \log a + b/b' (\log D/a')$$

where a and b are from the wet weight equation and a' and b' are from the dry weight equation. This equation was derived from simultaneous solution of the two above equations. The correlation coefficients are coincidently identical having r-values of 0.91. Satchell (1969) discusses conversion of fresh (wet) weight to dry weight. His regression analysis of the data from 44 specimens of *L. terrestris* gave the relation: 1 g dry weight = 5.5 g wet weight. His r-values or confidence limits were not given. Satchell's regression has been used in the last column of Table 2 to give a rapid comparison with European workers who have used wet (fresh) weight values in their publications. I strongly urge the use of dry weight values. Others have suggested the use of measuring biovolume as an alternative to biomass (Bouche, 1966).

Satchell (1967) reported 100 to 250 g/m^2 (wet weight) earthworm biomass using formaldehyde extraction. *Lumbricus terrestris* was the major species in the populations sampled. Until recently, the most quoted figure was Bornebusch's (1930) estimate of 61 g/m^2 from a *Quercus mull.* Satchell suggests that the earthworm biomasses of grassland and woodland soils are similar for similar soil types. He also points out that values for biomass are subject to large standard errors arising from seasonal and spatial variations. Raw (1959) using both hand sorting and formaldehyde in 23 apple orchard plots found densities of 848 per m^2 with an estimated biomass of 287 g/m^2 (wet weight). Satchell (1969) stated the average biomass of two *Lumbricus terrestris* populations was about 145 g/m^2. Reynolds (1970) reported wet weight biomass estimates ranging from 1.3 to 41.1 g/m^2 using formalin on forest habitats in Tennessee. In these Indiana woodlots the estimated biomass values ranged from a maximum of 280.28 g/m^2 on McCormick's Woods to a minimum of 26.29 g/m^2 on Black Locust. The average biomass for these six sites was 155.97 g/m^2. This maximum is 549% of the most frequently quoted figure of 61 g/m^2 (Bornebusch, 1930) for a typical woodland mull, 98% of Raw's value, and 112% of Satchell's 1967 maximum. But this maximum is 682% of Reynolds' 1970 maximum value. Many researchers for some time have suspected that Bornebusch's estimate was too low. Reynolds attributed his low biomass estimates to the absence of large species such as *Allolobophora longa* and *Lumbricus terrestris* and sampling during a dry period.

In the past, researchers have tried to superimpose European information and conclusions on North American conditions, but it is unjustified (Gates, 1970; Reynolds 1969 and 1970). Because of the impact of introduced European species on endemics and the wide spread distribution of a large and uniquely North American genus (*Diplocardia*) considerable research is required to truly evaluate the state of oligochaetes on this continent.

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LICHENS: BIOASSAY FOR AIR POLLUTION IN A METROPOLITAN AREA (NASHVILLE, TENNESSEE)

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Abstract

The high cost of buying and maintaining air pollution monitoring equipment has led to a search for better and less expensive ways of measuring pollution levels. Bioassay by means of lichens seems to have great potential for this purpose. The appropriateness of lichens for use as indicators of atmospheric pollution goes beyond matters of economy and encompasses the more important questions of how and why *living organisms* respond to atmospheric pollutants.

In this study corticolous lichens were utilized in calculating indices of atmospheric polluton (IAP's). IAP's were calculated for twelve stations along an east-west transect through the Nashville, Tennessee area. No significant correlations between 1970 pollution levels and IAP measurements was found. Thus, it was concluded that IAP's are probably of little use in monitoring pollution levels on a daily, weekly, or monthly basis. However, the low IAP's found near the center of the city and the comparatively high IAP's found in peripheral areas appear to be very definitely related to long-range effects of pollution in these areas.

Low variability in bark pH was correlated with an efficient buffering system. The source of the buffer chemicals appears to come from various external sources of particulate matter. The lack of correlation between bark pH and IAP suggests that pollutants selectively restrict the distribution of lichens by some method(s) other than by changing the pH of the substrate.

This and other studies show that corticolous lichens are sensitive to air pollution and quantitative measurements suggest their presence, coverage, and frequency can be used to bioassay pollution levels in metropolitan areas.

Introduction

The air pollution problems which have resulted from urbanization and industrialization are so well known as to preclude the need for documentation. Nevertheless, it seems appropriate to mention that Nashville ranks twentieth in sulfur dioxide levels (Bontrager, *personal communication*) and twenty-fifth in suspended particulate matter levels (U. S. Dept. of H. E. W., 1969) among American cities.

If certain lichen species can be identified which are reliable indicators of common aerial pollutants, then it logically follows that distributional studies of these

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