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Pesticidal Activity of Plant Extracts and a Mycoinsecticide (*Metarhrizium anisopliae***) on Cowpea flower Thrips and Leaves Damages in the Field**

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Authors' contributions

This work was carried out in collaboration between all authors. Author RBB designed the study, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Authors AN and ENN performed the statistical analysis of the study, language edited, read and approved the final version of the manuscript.

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ABSTRACT

The potentials of *Azadirachta indica*, *Boswellia dalzielii* aqueous extract and *Metarhizium anisopliae*, alone and/or in combination, as well as a reference insecticide Decis in controlling the cowpea flower thrips (*Megolurothips sjostedti)* were compared on two *Vigna unguiculata* varieties in the field. The field trials were arranged in a completely randomized block design with nine treatments, each of which was replicated four times. The nine treatments included a control, and the eight tested insecticide products. *Vigna unguiculata* plants were sprayed at flowering thrice with insecticide products at 5 days interval. Data assessment consisted of counting adults and thrips larvae population after three sprays, following up their dynamics, then estimating damages caused on leaves. All the tested insecticides significantly (p < 0.0001) reduced the populations and stabilized the dynamics of both adults and larvae thrips on the studied cowpea varieties. These bioinsecticides also contributed to the substantial reduction ($p = 0.0002$) of damages on cowpea leaves caused by thrips compared to the control treatment. The combination *M. anisopliae + A. indica + B. dalzielii* was the best of all treatments with nearly 90% reduction of these pests. The cowpea

variety B125 was more sensitive to the pest control than the local Bafia variety. As the outcome of the study, *Azadirachta indica*, *Boswellia dalzielii*, *M. anisopliae* and their combinations could be considered as potential natural insecticide in the management of thrips population on *V. Unguiculata* in the fields. This would increase *V. unguiculata* yield and free environmental pollution from synthetic insecticides.

Keywords: Azadirachta indica; Boswellia dalzielii; Metarhizium anisopliae; Vigna unguiculata; thrips.

1. INTRODUCTION

Agriculture is important for development in many countries in the world [1]. Therefore, productivity should be varied by cultivating crops with diversified incomes such as cowpea. Cowpea, *V. unguiculata* (L.) Walp, occupies an important place in the Sudano-Sahelian and Guinean savannah zones [2], whereit is grown for its high protein diet (20-25%) [3]. The seeds and leaves are used in a variety of dishes [4]. Cowpea also serves as animal fodder and can be used as green manure [5]. Cowpea contains 3400 calories and 230 g of protein per kg, twice as much as millet and sorghum [6], which are the main crops in Northern Cameroon. In addition to its nutritional qualities, cowpea improves the soil fertility by its ability to biologically fixing atmospheric nitrogen [7]. Cowpea contains 2 folds proteins than millet and sorghum [6], which are the main crops in northern Cameroon, where it is one of the most widely cultivated and consumed grain legumes [8].

However, cowpea cultivation is facing several problems, including fungal, bacterial and viral diseases [9], and insect pests that are responsible for much yield damages and losses [10]. Indeed, cowpea is almost attacked at all stages of its development, from the field to storage [11]. Potential insect pests in the field are flower thrips, *Megalurothrips sjostedti* Trybom (Thysanoptera, Thripidae), pod borers *Maruca vitrata* Fabricius (Lepidoptera, Pyralidae), and chinch bugs *Clavigralla tomentosicollis* Stal (Heteroptera, Coreidae) [12]. Of all these insect pests, *M. sjostedti* is the first to appear on plant [13]. They cause necrosis or even total destruction of flowers or flower buds, resulting in yield losses ranging from 20 to 70% [14-16]. It is therefore necessary to control these major insect pests of cowpea, in particular *M. sjostedti* if yield losses are to be reduced.

Previous studies have shown that increased cowpea yields is attributed to the use of synthetic insecticides [17-20], without which, thrips would considerably reduce the yield of this legume [2123]. Unfortunately, the use of these chemical pesticides is associated with many harmful effects [24]. Not only, do they have high costs, but also they acidify the soil in case of misuse, while their residues are toxic to non-target animals, in addition to resistance they develop to insect pests [25,26]. Moreover, they pollute surface and groundwater when they are washed out [27]. It would then be important to develop healthier and ecological control strategies to increase insect pest control and the productivity of crops while protecting our environment.

Extracts of many plants provide natural insecticides, and can therefore be used as a substitute for chemical insecticides. For this purpose, neem tree (*Azadirachta indica*) has been revealed as an insecticidal plant [28]. Its extract has been shown to be effective in controlling more than 400 harmful arthropods species [29]. Another plants namely *Boswellia dalzielii*, also called "incense tree" has been used as bio-insecticidal by Maffa women in the Farnorth Cameroon to protect stored food [30], and repels flies and mosquitoes [31]. These attributes make these two plants as potential alternatives to chemical pesticides.

The use of the enthomopathogenic fungus *M. anisopliae* against the flower thrips *M. sjostedti*was has been reviewed [32], and has shown insecticidal potential in controlling cowpea thrips [33]. From our basic knowledge, the combination of *Boswellia* and *Azadirachta* extracts to the entomopathogenic fungus *M. anisopliae* has not yet been tested against cowpea flower thrips. Therefore, in order to improve the protection of cowpea through the use of natural substances against insect pests, *A. indica, B. dalzielii* and *M. anisopliae* could be investigated.

In this work, we assess the influence of the application of *M. anisopliae* and the aqueous extracts of *A. indica* and *B. dalzielii* alone or in combination on the thrips population, and evaluate their contribution in reducing damages on cowpea leaves in the field.

2. MATERIALS AND METHODS

The experiment was carried out in the Guinean
Savannah agro-ecological zone (Dangagro-ecological Ngaoundéré). Trials were conducted for two consecutive years (2014 and 2015), and the field working dates are summarized in Table 4. Plant material consisted of two cowpea seeds varieties: the local Bafia multiplied locally during subsequent work, and the B125 provided by the Institute for Agricultural Research and Development (IRAD) Maroua. The B125 variety was an early maturity variety (75 days), whereas, the Bafia variety was an intermediate variety (85 to 95 days).

2.1 Experimental Layout and Treatments

Plants were grown on flat on (57.75 \times 25) m² surface. The experimental field was divided into two parts separated 4 m apart. The experimental plots representing the treatments were (4.5 × 1.5) m^2 for the B125 variety, and (4.5 \times 2.25) m^2 for the Bafia variety. According to the prescribed guidelines [34], seeds were planted at 50 cm between the lines, and 10 cm within the lines for the early variety, and 75 cm between, 20 cm within the lines for the intermediate variety. Insecticidal formulations were sprayed using four distinct manual gauge sprayers, each corresponding to a specific insecticidal product. For multi-product treatments, each component was sprayed separately. Treatments were applied early in the morning between 6 a.m and 8 a.m, 3 times at 5 days interval, as soon as the appearance of the first flower was noticed.

The experimental design applied to each variety was fully randomized, consisting of 9 treatments, each of which was repeated 4 times. The different treatments were: T1, negative control representing plots that received no insecticidal treatment; T2, plots treated with aqueous *A. indica* leaves extract; T3, plots treated with aqueous *B. Dalzielii* leaves extract; T4, plots treated with *M. anisopliae* formulation; T5, plots treated with the combination of *M. anisoplia + A. indica*; T6, plots treated with the combination *M.*

anisopliae + B. dalzielii; T7 plots treated with the combination *A. indica + B. dalzielii*; T8, plots treated with the combination of the three bioinsecticides *M. anisopliae + A. indica + B. dalzelii*; T9, the plots treated with the chemical insecticide Decis®.

2.2 Formulation of Insecticides Products

The aqueous extract based *A. Indica* leaves was obtained following the method recommended by Sahel People Service (Data Sheet 2). According to this method, 5L of solution was obtained by macerating 1kg of *A. indica* fresh leaves in water. The resulting concentrated maceratewas then diluted to 10% with water and filtered through a 0.4 mm mesh tissue, for a working concentration of 20g/L. For the formulation of the *B. dalzielii* leave insecticide, the aqueous extract method of the *A. Indica* leaves was applied. The *M. anisopliae* based solution was obtained using the formulation described [33], that requires the mixture of 50 g of *M. anisopliae* with 700 ml of kerosene and 300 ml of cotton oil (Diamaor. For this work, *M. anisopliae* was prepared at a concentration of 10g/L. The myco-insecticide *M. anisopliae* originated from IITA Cotonou-Benin, while Deltamethrin-based synthetic insecticide (Decis®) purchased from the phytosanitary store was obtained by diluting 3 mL of Decis® in 15 L of water [33].

2.3 Assessed Parameters

The average number of thrips per flower (larvae and adults) and the average number of holes per leaf were assessed. The determination of thrips population on cowpea flowers was performed at flowering-pod-forming stage, just after the three applications of the treatments [35].

A total of 25 blooming flowers randomly taken per plot at 5 days interval (5 flowers per day) were placed seperately in 25ml plastic vials into 50% alcohol as described [36]. This number of flowers was a realistic sample size for statistical analysis [10]. To reduce disturbance of insects,

Table 1. Cropping calender

the field was not visited before data collection scheduled from 08:00 to 10:30. Flowers were dissected in the laboratory where *M. sjostedti* larvae and adults were counted separately under a binocular stereomicroscope [37]. The number of holes on leaves was evaluated on 10 random leaves per plot on plants in the middle rows. This assessment of leaf damages was performed after thrips counts on day 6 after the last spraying.

2.4 Statistical Analysis

The statistical analysis of data was carried out using the SAS software. The density of thrips (larvae and adults), thrips population dynamics (adults and larvae) and the number of holes per leaf, were subjected to the analysis of variance (ANOVA) to split the averages. The Student-Newman-Keuls test was used to compare the different treatments.

3. RESULTS

3.1 Influence of Insecticide Formulations on the Adult Thrips Population Density

In 2014, insecticide treatments significantly ($p <$ 0.0001) reduced the density of adult thrips of the B125 variety compared to the negative control (Fig. 1). Among the bio-insecticidal treatments, *M. anisopliae* had a more pronounced effect on adult thrips than others taken separately or in combination. It reduced the adult thrips density by nearly 90%, as much as the synthetic insecticide Decis taken as a positive control. Despite the reduction in the population of adult thrips by 50% *A. indica* was the least effective bio-insecticide treatment. As for the Bafia variety, insecticide treatments significantly reduced the density of adult thrips compared to the negative control (p<0.0001). *M. anisopliae* and its different combinations consistently impacted the adult thrips population than other bio-insecticide treatments. Once again, *A. indica* was the less effective treatment. All bio-insecticides applied to cowpea variety B125 significantly (p <0.0001) reduced the density of adult thrips compared to the negative control in 2015, but Decis was the most effective. The adult thrips population observed on cowpea local Bafia variety was greater (p < 0.0001) on *A. indica* treated plants than other treatments. Generally, the density of adult thrips population was lower in cowpea B125 variety during the 2014 growing season

than in 2015. The opposite was true for the Bafia variety.

3.2 Influence of Insecticide Formulations on the Larvae Thrips Population Density

Similar to the density of adult thrips, bioinsecticidal treatments positively affected the density of larvae thrips. The results of Fig. 2 indicate that the synthetic insecticide Decis contributed to the total reduction of larvae thrips population in 2014 for both cowpea varieties B125 and Bafia. With a 90% reduction of larvae, *M. anisopliae*, *M. anisopliae + A. indica + B. dalzielii* were the most effective bioinsecticides. *A. indica, B. dalzielii* and their combination, were the least effective treatments with a 50% reduction of larval density. In 2015, the impact of Decis did not completely reduce the density of larvae thrips. On the B125 cowpea variety, bio-insecticides significantly reduced the larvae thrips density by 30% compared to the negative control, except for *B. dalzielii* treatment which had as many thrips larvae as the negative control ($p < 0.0001$). On the Bafia variety, unlike the *A. indica* treatment, which had more thrips than the negative control, all bio-insecticides significantly reduced the density of larvae thrips compared to the negative control (p <0.0001). The combination *M. anisopliae* + *A. indica* + *B. dalzielii* which was the most effective treatment with 90% reduction, acted like Decis. The density of larvae thrips was lower on B125 variety during the 2014 cropping season than that of 2015. This was the opposite with the Bafia variety.

3.3 Influence of Insecticide Formulations on the Population Dynamics of Adult Thrips

As far as the B125 variety is concerned, relative stability of the population density of adult thrips for various insecticidal treatments was observed in 2014 (Fig. 3). This stability was disrupted by a total decrease in the density of adult thrips on day 4, followed by an increase in day 5, more pronounced in treatments *A. indica* leaves extract. *M. anisopliae* + *A. indica* + *B. dalzielii* was the composite treatments that had the most stable population dynamics and the least amount of adult thrips. As for Bafia variety, the density of adult thrips decreased gradually until day 3 before a fluctuation on day 4, followed by a stabilization. The aqueous extract from *A. indica* leaves maintained the adult thrips population low

over time. In 2015, the population dynamics of adult thrips were rather stable in different insecticide treatments, unlike the negative control (Fig. 4). However, treatment Decis kept the adult thrips population as low as possible, with the most stable dynamics. The relative stability observed in most treatments of the Bafia variety was disrupted in *M. anispliae* + *A. indica* + *B. dalzielii* and *A. indica* treatments. As with the B125 variety, it was the Decis that kept the population of adult thrips low over time.

Fig. 1. Density of adult thrips on cowpea B125 and Bafia varieties as influenced by insecticidal treatments in 2014 (A) and 2015 (B)

T: negative control; A: A. indica; B: B. dalzielii; M: M. anisopliae; M+B: M. anisopliae + B. dalzielii; M+A: M. anisopliae + A. indica; A+B: A. indica + B. dalzielii; M+A+B: M. anisopliae + A. indica + B. dalzielii; D. Chemical *insecticide Decis.Bars affected with the same letters are notdifferent at5% level of significantly(Student– Newman–Keuls test)*

Fig. 2. Density of larvae thrips on cowpea B125 and Bafia flowers as influenced by insecticidal treatments in 2014 (A) and 2015 (B)

T: negative control; A: A. indica; B: B. dalzielii; M: M. anisopliae; M+B: M. anisopliae + B. dalzielii; M+A: M. anisopliae + A. indica; A+B: A. indica + B. dalzielii; M+A+B: M. anisopliae + A. indica + B. dalzielii; D: Chemical insecticide Decis. Bars affected with the same letters are not different at 5% level of significantly (Student-*Newman–Keuls test)*

3.4 Influence of Insecticide Formulations on Population Dynamics of Thrips Larvae

The density of larvae thrips in 2014 was relatively stable on B125 variety flowers (Figure 5). Similar to what was observed on adults, this stability was disrupted on day 4 by a sudden decrease of larvae density, before an increase on day 5. Among the bio-insecticides, treatments *M. anisopliae* + *A. indica* + *B. dalzielii* was the most effective with prolonged action. Decis was the treatment that had the lowest number of larvae stable in dynamics. Concerning the Bafia variety, the population dynamics of larvae declined gradually before growing up and

stabilized. It was *A. indica* based extract that had the greatest number of larvae. Treatment Decis as synthetic insecticide was the most effective treatment.

In 2015, the population dynamics of larvae thrips remained relatively stable in both Bafia and B125 varieties, with *B. dalzielii* treatment not having too much effect on larvae (Figure 6). Decis was the most effective insecticide treatment. The same was true for the cowpea Bafia variety, but with *A. indica* the least effective treatment on larvae.

influenced by insecticidal treatments in 2014

T : negative control; A: A. indica; B: B. dalzielii; M: M. anisopliae; M+B: M. anisopliae + B. dalzielii; M+A: M. anisopliae + A. indica; A+B : A. indica + B. dalzielii; M+A+B : M. anisopliae + A. indica + B. dalzielii; D. Chemical *insecticide Decis*

Evaluated as an average number of holes bore on leaves, leaves damages varied from one treatment to another. The results obtained after spraying cowpea B125 and Bafia varieties during the 2014 and 2015 cropping seasons with different insecticide formulations are shown in Figure 7. Treatments *M. anisopliae* and *M. anisopliae* + *B. dalzielii* protected the leaves better than others, for which damages were similar to those occurring after application of the synthetic insecticide Decis. On the cowpea Bafia variety, the bio-insecticides treatments significantly ($p = 0.0002$) protected the cowpea leaves from perforation of holes on leaves better than the negative control. All the bio-insecticides protected the leaves, except treatment *A. indica,* which was less efficient in protecting leaves. In contrast, in 2015, the two cowpea varieties B125 and Bafia showed no significant difference between treatments ($p = 0.1520$ for the B125 variety, $p = 0.2110$ for the Bafia variety). The various bio-insecticides and the synthetic insecticide Decis equally and significantly protected the cowpea plants better than the negative control.

4. DISCUSSION

Various biopesticides used as treatments in this study have influenced the investigated parameters. Adult thrips density was higher in the 2014 cropping season than in 2015. In a related study, more thrips were found in cowpea flowers during the first than the second cropping season, indicating that the effect of insecticidal products may vary with time and space [33]. In 2014, thrips leaching caused by rainfall [38], was more frequent during flowering of cowpea B125 variety, and reduced the density of adult thrips compared to that of Bafia variety, which had a more longer growing cycle. After rainfall, the cowpea thrips density dropped down in all the treatments, similar to recent observations that highligthed that rainfalls, high speed winds can displace insects from their location points in plant organs [39,40]. On the other hand, it has been reported that during heavy rain, insect larvae are washed out from the host plants [41], thus reducing their populations. From the various bio-insecticides tested, the reduced efficacy of neem-based extract in the field was evidenced, in agreement with low efficiency of aqueous neem extract in the field against aphids

and acarians [42]. However, with its systemic action, neem extract was proven to be more efficient on certain aphids, lepidoptera, diptera, orthoptera, hymenoptera, and larvae of coleoptera [28,43]. Despite its insecticidal properties [29], it was the least effective insecticide treatment duning this study. These results are in line with other findings that have revealed the least effeiciency of neem products in the field [24]. They seem to act better on stored pests through azadirachtin, the main pesticidal component of neem extracts specially found more concentrated in neem seed extract, and possessing feeding deterrent, repellent, toxic, as wll as growth disruption properties against numerous pest species [44]. Difference in insecticidal efficacy was reported to vary between one part of plant and the other, depending on the level of concentration of the anti-insect or anti-feedant compounds present therein [45]. In 2015, with less frequent rains during the flowering period, *A. indica* extract was as efficient on cowpea B125 variety as other bioinsecticides. In addition to its insecticidal activity *B. dalzielii* extract is rich in gum [31,46], which is responsible for its viscosity. This viscosity is an adhesive factor that would have accounted for the reduced thrips density much more than that of*A. indica* extract on cowpea B125 variety in 2015. Adhesion has been reported to be an important factor in the effectiveness of insecticides [47]. In a similar research, highest adult mortality (43.11%) of *Callosobruchus maculatus* was observed in cowpea seeds treated with 10% (w/w) *B. dalzielii* leaf powder, followed by stem back (25.00%) [48].

The effectiveness of the *M. anisopliae* treatment was somewhat boosted by other accompanying components (kerosine, cottonseed oil) which also have been reported to deserve insecticidal properties [49]. Moreover, they promote the apprpriate application of the extract for the direct contact with thrips [47]. On the overall, this has enabled the myco-insecticide to be more effective than *A. indica* and *B. dalzielii* extracts, and equallly effective as the synthetic insecticide Decis in 2014 on both cowpea varieties.

In the combined treatments, the synergistic activity of components has improved its effectiveness compared to individual treatments. Treatment *A. indica*+*B. dalzielii* extracts by this synergistic effect would therefore, have reduced thrips density better than *B. dalzielii* extract alone, or other treatments in 2014. *M. anisopliae* and the other biopesticides used in this work

have synergistically acted in reducing the thrips
population. Decis, the broad-spectrum broad-spectrum insecticide, was more effective than the various bio-insceticides [24,35]. The insecticidal activity of bioproducts on adults thrips was similar to the one on larvae [33], but with a more pronounced effect on larvae that are depleted in cuticle, the primary protective barrier of insects [50].

Fig. 4. Population dynamic of adult thrips on cowpea B125 (A) and Bafia (B) varieties as influenced by insecticidal treatments in 2015

T: negative control; A: A. indica; B: B. dalzielii; M: M. anisopliae; M+B: M. anisopliae + B. dalzielii; M+A: M. anisopliae + A. indica; A+B: A. indica + B. dalzielii; M+A+B: M. anisopliae + A. indica + B. dalzielii; D. Chemical *insecticide Decis*

Fig. 5. Population dynamic of larvae thrips on cowpea B125 (A) and Bafia (B) varieties as influenced by insecticidal treatments in 2014

T : negative control; A: A. indica; B: B. dalzielii; M: M. anisopliae; M+B: M. anisopliae + B. dalzielii; M+A: M. anisopliae + A. indica; A+B : A. indica + B. dalzielii; M+A+B : M. anisopliae + A. indica + B. dalzielii; D. Chemical *insecticide Decis.*

The population dynamics of thrips was moreless stable in different insecticide treatments. All insecticides had a similar effect on the population dynamics of thrips, close to the reported results on the effect of biofertilizers and a mycoinsecticide on the management of cowpea flower thips in Cameroon [33]. This thrip dynamic was lower on cowpea B125 than on the Bafia variety

in 2014. The frequency and abundance of the rains during the flowering period of B125 variety would have resulted in leaching thrips. The wind and water runoff would also have transported thrips [38]. As for the various insecticidal treatments, *A. indica* extract had the highest dynamics on B125 in 2014 due to its reduced field efficiency [24]. Other insecticidal treatments

with a higher viscosity (.*B Dalzielii*), or adhesivepromoting components (*M. anisopliae*) were less washed out by rainfall. The highest thrips densities (day 1) on *M. anisopliae* treatment, and day 2 on the Decis treatment were due
to the unequal distribution of thrips unequal distribution of thrips

T : negative control; A: A. indica; B: B. dalzielii; M: M. anisopliae; M+B: M. anisopliae + B. dalzielii; M+A: M. anisopliae + A. indica; A+B: A. indica + B. dalzielii; M+A+B: M. anisopliae + A. indica + B. dalzielii; D: Chemical *insecticide Decis*

T: negative control; A: A. indica; B: B. dalzielii; M: M. anisopliae; M+B: M. anisopliae + B. dalzielii; M+A: M. anisopliae + A. indica; A+B: A. indica + B. dalzielii ; M+A+B: M. anisopliae + A. indica + B. dalzielii; D. Chemical insecticide Decis. Bars affected with the same letters are not different at 5% level of significantly (Student-*Newman–Keuls test).*

population in the field during these days. The total leaching of thrips by rain on day 4 completely eliminated thrips as recently revealed [38]. On cowpea Bafia variety, although the viscosity of *B. dalzielii* extract enabled stabilization of thrips population better than *A. indica* extract, *B. dalzielii* extract was less efficient than *M. anisopliae* and Decis.

The insecticidal activities of treatments have allowed reduction of damages on cowpea leaves of both B125 and bafia varieties in 2014 and 2015. Cottonseed oil, which favors the spreading and fixation of *M. anisopliae*, made the leaves obsolete. The viscosity of *B. dalzielii* [46] might have enabled better protection of cowpea B125 leaves from thrip damages than other treatments

in 2014. The combined treatment *A. indica*+*B. dalzielii*+*M. anisopliae* have effectively controlled thrips population in 2015. The presence of metabolites (such as azadirachtin in neem) gives the plant materials their insecticidal ability. These compounds, upon consumption by insects led to poisoning effect or, when in contact, probably blocked their respiration passages or injured the insect cuticle resulting in sudden death [51]. Refering to their efficacy, the botanical extracts used in this study are suitable for integrated pest management because of their low toxicity to non target organisms, easy preparation and compatibility with other bio-products.

5. CONCLUSION

This work was conducted to seek for sustainable ways of improving the control of the cowpea flower thrips *M. sjostedti* in the field using bioinsecticides. As the outcomes, the aqueous extracts of *A. indica* and *B. dalzielii*, the mycoinsecticide *M. anisopliae*, and their various combinations have resulted in reducing the population density of thrips (adults and larvae), and stabilizing their dynamics in the field. These bio-insecticides also contributed to the reduction of damages on leaves, and could therefore be proposed to substitute the commonly used synthetic insecticides for a sustainable monitoring of this major cowpea pest in field.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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