

IMMUNOLOGIC REACTIVITY TO WORK-RELATED AIRBORNE ALLERGENS IN PEOPLE OCCUPATIONALLY EXPOSED TO DUST FROM HERBS

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Abstract: A group of 150 people occupationally exposed to dust from herbs were examined. The examined group consisted of 47 thyme farmers, 32 chamomile farmers, 31 sage farmers and 40 workers of herbs processing industry. As a reference group, 50 urban dwellers, not exposed to any kind of organic dust, were examined. Skin prick tests and precipitin tests were conducted with, respectively, 4 and 11 microbial antigens associated with organic dust. Both skin and precipitin tests were also conducted with herbal extracts of chamomile and sage. Precipitin tests were carried out with sera not concentrated and sera 3-fold concentrated. Tests for inhibition of leukocyte migration (MIF) were also conducted with 4 microbial antigens. People occupationally exposed to dust from herbs showed a higher frequency of positive skin reactions to microbial antigens compared to the reference group. The results of precipitin test also revealed greater reactivity to the environmental microbial antigens in the examined group, compared to the reference group. The highest frequency of positive results was noted with the antigen of *Pantoea agglomerans* (30.6% with sera not concentrated and 48.3% with sera 3-fold concentrated) - the difference compared to the reference group (12.0%) was highly significant ($p < 0.01$). The frequencies of positive results of MIF test in the examined group were high with all antigens tested: *Arthrobacter globiformis* (12.6%), *Pantoea agglomerans* (11.1%), *Saccharopolyspora rectivirgula* (17.0%), *Aspergillus fumigatus* (13.3%), and, compared to the reference group with no positive result for any antigen, all the differences were significant ($p < 0.05$). In conclusion, the frequency of positive allergological reactions to airborne microorganisms was high in people occupationally exposed to dust from herbs and suggests a potential role of microbial allergens in the pathogenesis of work-related health disorders among herb workers. The risk of sensitization seems to be greatest among thyme farmers, who showed the highest positive response. The results confirmed the particular allergenic importance of Gram-negative bacterium *Pantoea agglomerans*.

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INTRODUCTION

Herbs are a heterogenous group of several thousands plant species, which are used for production of medicines, cosmetics and spices, or for ornamental purposes.

According to Robbers and Tyler, herbs are non-woody plants that die to the ground at the end of the growing season and are sources of crude drugs utilized for the treatment of diseases, often of a chronic nature, or to attain or maintain a condition of improved health [30].

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Herbs are nowadays produced and used in much larger quantities. By 1999, the value of the world phytomedicinals market was assessed as over 20 billion USD and the annual world production of herbs as 400,000 tons. The value of the European phytotherapeutic market alone increased 5-fold during the years 1992–1999, from 1.4–7 billion USD [3, 27].

As a result of the growth of herbal production, the number of people occupationally exposed to dust from herbs and herb allergens has also increased. In Poland alone, about 100,000 farmers cultivating and processing herbs and over 2,500 workers of herb processing industry are exposed to herb allergens.

Harmful effects of exposure to dust from herbs have been demonstrated by numerous authors [12, 13, 14, 21, 23, 38, 39]. A high proportion of herb farmers and workers of herb processing industry complain of work-related respiratory, skin, conjunctival and general symptoms [12, 13, 14]. Adverse effects have been described following long time exposure to dust from herbs in workers of herb processing industry [39], and increased reactivity of the exposed workers to microorganisms associated with dust from herbs [12]. Clinical cases of occupational asthma [21], allergic conjunctivitis [37] and systemic allergic reactions [1] caused by herbal allergens have been reported. Occupational exposure to dust from thyme may cause allergic alveolitis [23] and airborne contact dermatitis [38].

The aim of the present study was to determine the reactivity of herb farmers and workers of herb processing industry to various allergens associated with herb dust, with particular attention to epiphytic microorganisms, known as etiologic agents of respiratory disorders due to exposure to organic dusts [4, 9, 19, 20, 22, 26, 32].

MATERIALS AND METHODS

Examined population. A group of 150 people occupationally exposed to dust from herbs, aged 42.8 ± 11.7 yrs (range 15–74 yrs) were examined. The examined group consisted of 47 farmers cultivating thyme (*Thymus vulgaris* L.), 32 farmers cultivating chamomile (*Matricaria chamomilla* L.), 31 farmers cultivating sage (*Salvia officinalis* L.) and 40 workers of herb industry employed in a big facility processing various herbs. The group comprised 66 males and 84 females.

As a reference group, 50 urban dwellers, not exposed to any kind of organic dust were examined. The group consisted of 24 males and 26 females, aged 37.4 ± 12.1 yrs (range 20–65 yrs).

Allergological tests. Skin tests, agar-gel precipitation tests and tests for specific inhibition of leukocyte migration were applied in the groups of herb workers and controls using the antigens of microorganisms associated with organic dusts, and antigens of herbs. Antigens of the following microorganisms, occurring commonly in the agricultural working environment of eastern Poland and reported as causative agents of work-related respiratory

disorders, were used for the production of antigens (allergens):

- Gram-negative bacteria of the species *Acinetobacter calcoaceticus*, *Alcaligenes faecalis* and *Pantoea agglomerans* (syn.: *Erwinia herbicola*, *Enterobacter agglomerans*);
- Gram-positive non-branching bacteria of the species *Arthrobacter globiformis* and *Bacillus subtilis*;
- Actinomycetes of the species *Streptomyces albus*, *Saccharopolyspora rectivirgula* (syn.: *Micropolyspora faeni*, *Faenia rectivirgula*) and *Thermoactinomyces vulgaris*;
- Fungi of the species *Aspergillus candidus*, *Aspergillus fumigatus* and *Penicillium citrinum*.

Sage (*Salvia officinalis*) and chamomile (*Matricaria chamomilla*) extracts were used as herbal antigens (allergens).

In all tests, lyophilised saline extracts of bacterial or fungal mass, produced in the Institute of Agricultural Medicine in Lublin, were used as antigens. In the case of mesophilic, non-branching bacteria the mass was harvested from nutrient agar cultures while in the case of actinomycetes and fungi the mass was harvested from sugar broth cultures. The mass was then homogenised and extracted in saline (0.85% NaCl) in the proportion 1:2 for 48 hrs at 4°C, with intermittent disruption of cells by 10-fold freezing and thawing. Afterwards, the supernatant was separated by centrifugation, dialysed against distilled water for 24 hrs, concentrated by evaporation to 0.1–0.15 of previous volume and lyophilised [26, 34, 35].

Herbal antigens were obtained by extraction of minced and defatted sage leaves and chamomile flowers in Coca's solution in the proportion 1:10 (w/v) [36], dialysis against distilled water and lyophilisation.

Skin tests were carried out by prick method with the antigens of *Pantoea agglomerans* (syn.: *Erwinia herbicola*, *Enterobacter agglomerans*), *Streptomyces albus*, *Saccharopolyspora rectivirgula*, *Aspergillus fumigatus*, and herbal extracts of chamomile (*Matricaria chamomilla*) and sage (*Salvia officinalis*). The antigens were dissolved in saline (P.B.S., Biomed, Kraków, Poland) at the concentration of 5 mg/ml. The diluted allergens were sterilised by filtering and checked for sterility and lack of toxicity. The test was performed on the forearm with the antigenic extracts, P.B.S. as a negative control, and histamine dihydrochloride (Allergopharma, Reinbek, Germany) as a positive control. The test sites were observed after 20 min. Wheal and/or erythema reactions of 3 mm or more in diameter were regarded as positive [35].

The agar-gel precipitation test was performed by Ouchterlony double diffusion method in purified 1.5% Difco agar with the antigens of *Acinetobacter calcoaceticus*, *Alcaligenes faecalis*, *Arthrobacter globiformis*, *Bacillus subtilis*, *Pantoea agglomerans*, *Streptomyces albus*, *Saccharopolyspora rectivirgula*, *Thermoactinomyces vulgaris*, *Aspergillus candidus*, *Aspergillus fumigatus*, *Penicillium citrinum*, and herbal extracts, either of chamomile (in chamomile farmers and industry workers) or sage (in sage farmers and industry workers). The worker's serum was placed in the central well and

Table 1. Frequency of positive skin reactions to antigens associated with herb dust in herb workers and referents.

Antigen	Persons showing positive reactions (number, percent)					
	Thyme farmers N = 47	Chamomile farmers N = 29 [†]	Sage farmers N = 32	Herb industry workers N = 40	Total herb workers N = 148 [‡]	Reference group N = 50
<i>Pantoea agglomerans</i>	7 (14.9%)*	0	1 (3.1%)	4 (10.0%)	12 (8.1%)	1 (2.0%)
<i>Saccharopolyspora rectivirgula</i>	4 (8.5%)*	0	1 (3.1%)	3 (7.5%)	8 (5.4%)	0
<i>Streptomyces albus</i>	2 (4.3%)	0	0	1 (2.5%)	3 (2.0%)	1 (2.0%)
<i>Aspergillus fumigatus</i>	2 (4.3%)	1 (3.4%)	1 (3.1%)	1 (2.5%)	5 (3.4%)	1 (2.0%)
Chamomile extract (<i>Matricaria chamomilla</i>)	N.t.	1 (3.2%)	3 (9.4%)*	2 (5.0%)	6 (5.8%)	0
Sage extract (<i>Salvia officinalis</i>)	N.t.	0	0	3 (7.5%)	3 (2.9%)	0

[†]With herb extracts 31 farmers were tested; [‡]With herb extracts a total of 103 workers were tested; *significantly greater compared to reference group, $p < 0.05$; N.t. = not tested.

antigens in the peripheral wells. The antigens were dissolved in saline at the concentration of 30 mg/ml. Each serum was tested twice: not concentrated, and 3-fold concentrated, for the detection of low levels of precipitins. The plates were incubated for 6 days at room temperature, then washed in saline and 5% sodium citrate solution (to prevent false positive reactions), and stained with azocarmine B [29, 34, 35].

Test for inhibition of leukocyte migration in the presence of specific antigen was performed by the whole blood capillary microculture method according to Bowszyc *et al.* [2] with the antigens of *Arthrobacter globiformis*, *Pantoea agglomerans*, *Saccharopolyspora rectivirgula* and *Aspergillus fumigatus*. Patient's blood and Parker's culture medium were added in the volumes of 0.5 ml and 0.12 ml, respectively, to two silicon test tubes. Then, 0.12 ml of the antigen solution in the concentration of 100 µg/ml was added to one tube, while to the other 0.12 ml of the diluent (P.B.S.) as a control. Both suspensions were incubated for 30 min at room temperature and thereafter distributed to heparinised glass capillary tubes 75 × 1 mm. Capillary tubes were sealed at both ends with the 4:1 mixture of paraffin and vaseline, centrifuged for 10 min at 1,500 rev/min and fastened tangentially on microscopic slides with sticky tape at an angle of 10°. The microcultures obtained were incubated for 4 hrs at 37°C in a humid chamber. The leukocyte migration distances, visible as distinct white zones, were measured under a binocular microscope. The results were expressed as a migration index (MI), i.e., the ratio of the mean migration distance of leukocytes in microcultures with antigen, to the analogical distance in microcultures without antigen. The test was considered as positive at the MI equal to 0.790 or lower.

Statistical analysis. Distribution of variables was checked for normality by Shapiro-Wilk test. Depending on the result, variables showing normal distribution were analysed by Student's *t*-test and Pearson's test for correlation while others were analysed by Wilcoxon test, Mann-Whitney U test and Spearman test for correlation.

For the analysis of discrete variables the homogeneity chi-square test was used. The $p < 0.05$ level was considered significant. The statistical analysis was carried out with the use of the StatisticaTM ver. 4.5 package (Statsoft©, Inc., Tulsa, Oklahoma, USA).

RESULTS

Skin prick test. Herb workers showed positive reactions to all tested antigens at a frequency of 2.0–8.1% (Tab. 1). The prevalence of positive skin reactions was higher in herb workers than in the reference group but the differences were significant only in few cases (Tab. 1). The highest noted frequencies of positive skin response were to the antigens of Gram-negative bacterium *Pantoea agglomerans* and thermophilic actinomycete *Saccharopolyspora rectivirgula*, in particular among thyme farmers who reacted significantly more frequently than referents ($p < 0.05$).

Agar gel precipitation test. The prevalence of positive precipitin reactions is shown in Table 2. Very high percentages of positive reactions to the antigen of *Pantoea agglomerans* were noted in the group of herb workers: 30.6% with not concentrated sera and 48.3% with 3-fold concentrated sera (Tab. 2). These values were significantly greater compared to the reference group ($p < 0.01$). The highest frequencies of positive precipitin reactions to *Pantoea agglomerans* were found in thyme farmers and workers of herb processing industry ($p < 0.01$ compared to reference group).

In thyme farmers high frequencies of positive precipitin reactions were also found to the antigens of *Alcaligenes faecalis* and *Aspergillus fumigatus*, with 3-fold concentrated sera significantly greater compared to the reference group ($p < 0.05$).

In sage farmers and chamomile farmers high frequencies of positive precipitin reactions to *Bacillus subtilis* were obtained, with 3-fold concentrated sera significantly greater compared to the reference group ($p < 0.05$). In sage farmers, a high prevalence of precipitin

Table 2. Frequency of positive precipitin reactions to antigens associated with herb dust in herb workers and referents (A - sera not concentrated, B - sera 3-fold concentrated).

Antigen	Persons showing positive reactions (number, percent)											
	Thyme farmers N = 46		Chamomile farmers N = 29		Sage farmers N = 32		Herb industry workers N = 40		Total herb workers [#] N = 147		Reference group N = 50	
	A	B	A	B	A	B	A	B	A	B	A	B
<i>Acinetobacter calcoaceticus</i>	0	4 (8.7%)	0	0	1 (3.1%)	3 (9.4%)	1 (2.5%)	1 (2.5%)	2 (1.4%)	8 (5.4%)	2 (4.0%)	2 (4.0%)
<i>Alcaligenes faecalis</i>	10 (21.7%)	13 (28.3%)*	0	1 (3.4%)	0	0	0	0	10 (6.8%)	14 (9.5%)	4 (8.0%)	4 (8.0%)
<i>Arthrobacter globiformis</i>	0	1 (2.2%)	0	0	0	0	0	0	0	1 (0.7%)	0	0
<i>Bacillus subtilis</i>	0	1 (2.2%)	0	5 (17.2%)*	4 (12.5%)*	5 (15.6%)*	0	0	4 (2.7%)	11 (7.5%)	0	0
<i>Pantoea agglomerans</i>	16 (34.8%)**	26 (56.5%)**	4 (13.8%)	9 (31.0%)*	11 (34.4%)*	14 (43.8%)**	14 (35.0%)**	22 (55.0%)**	45 (30.6%)**	71 (48.3%)**	6 (12.0%)	6 (12.0%)
<i>Saccharopolyspora rectivirgula</i>	0	1 (2.2%)	1 (3.4%)	1 (3.4%)	0	0	1 (2.5%)	1 (2.5%)	2 (1.4%)	3 (2.0%)	0	0
<i>Streptomyces albus</i>	0	2 (4.3%)	0	0	2 (6.3%)	4 (12.5%)*	0	0	2 (1.4%)	6 (4.1%)	0	0
<i>Thermoactinomyces vulgaris</i>	0	1 (2.2%)	0	0	1 (3.1%)	1 (3.1%)	0	1 (2.5%)	1 (0.7%)	3 (2.0%)	0	0
<i>Aspergillus candidus</i>	0	0	0	1 (3.4%)	0	0	0	0	0	1 (0.7%)	0	0
<i>Aspergillus fumigatus</i>	7 (15.2%)	13 (28.3%)*	0	0	0	0	0	0	7 (4.8%)	13 (8.8%)	4 (8.0%)	4 (8.0%)
<i>Penicillium citrinum</i>	1 (2.2%)	2 (4.3%)	0	0	0	1 (3.1%)	0	0	1 (0.7%)	3 (2.0%)	0	0
Chamomile extract (<i>Matricaria chamomilla</i>)	N.t.	N.t.	0	0	N.t.	N.t.	0	1 (2.5%)	0	1 (1.4%)	0	0
Sage extract (<i>Salvia officinalis</i>)	N.t.	N.t.	N.t.	N.t.	0	0	N.t.	N.t.	0	0	0	0

[#] A total of 69 workers were tested with chamomile extract and a total of 32 workers with sage extract; N.t. = not tested; **, * : significantly greater compared to reference group; * p < 0.05, ** p < 0.01.

reactions to *Streptomyces albus* was also noted with concentrated sera, significantly greater than in the reference group (p < 0.05).

The positive precipitin response to other microbial antigens and to herbal extracts was low or absent (Tab. 2).

Test for specific inhibition of leukocyte migration.

In the group of herb workers, high frequencies of positive reactions in the range of 11.1–17.0% were noted with the antigens tested (Tab. 3). The mean values of migration index (MI) in the presence of all antigens were significantly smaller in total herb workers compared to the reference group (p < 0.001) while the percentages of positive reactants were significantly greater (p < 0.05). Moreover, when comparing the results obtained in particular subgroups of herb workers with those in the reference group, significant differences were noted in most cases (Tab. 3). The highest frequencies of positive MIF reactions were noted in thyme farmers, especially

with *Arthrobacter globiformis* (26.5%) and *Saccharopolyspora rectivirgula* (23.5%). In both cases the differences versus reference group were highly significant (p < 0.01).

DISCUSSION

The frequency of positive skin prick test reactions with all tested antigens was higher in the group exposed to herb dust than in the reference group. Nevertheless, the prevalence of positive skin prick reactions was lower compared to farmers exposed to other dusts of plant origin [34, 35]. The frequencies of positive skin reactions to microbial antigens and herb extracts were also lower compared to those obtained by Dutkiewicz *et al.* [12] in the workers of herb processing factory with the use of more sensitive but less specific intradermal test. The prevalence of positive skin reactions to sage extract in the examined group of herb industry workers (7.5%) was

Table 3. Results of the test for inhibition of leukocyte migration in the presence of specific antigen in herb workers and referents.

Antigen	Value	Thyme farmers N = 34	Chamomile farmers N = 29	Sage farmers N = 32	Herb industry workers N = 40	Total herb workers N = 135	Reference group N = 50
<i>Arthrobacter globiformis</i>	MI (mean ± SD)	0.9242±0.1278 ^c	0.9133±0.0861 ^c	0.8832±0.0573 ^c	0.9083±0.0619 ^c	0.8987±0.0907 ^c	1.0152±0.0978
	Positive reactions (n, %)	9 (26.5%) ^{**}	2 (6.9%)	5 (15.6%) [*]	2 (5.0%)	17 (12.6%) [*]	0
<i>Pantoea agglomerans</i>	MI (mean ± SD)	0.9340±0.1206 ^b	0.8758±0.1647 ^c	0.8722±0.0605 ^c	0.9107±0.0677 ^c	0.9040±0.0885 ^c	0.9947±0.1045
	Positive reactions (n, %)	5 (14.7%) [*]	3 (10.3%)	5 (15.6%) [*]	2 (5.0%)	15 (11.1%) [*]	0
<i>Saccharopolyspora rectivirgula</i>	MI (mean ± SD)	0.9111±0.1448 ^b	0.9041±0.0925 ^c	0.8501±0.1655 ^c	0.9199±0.0865 ^c	0.9051±0.1024 ^c	1.0008±0.0098
	Positive reactions (n, %)	8 (23.5%) ^{**}	6 (20.7%) ^{**}	4 (12.5%) [*]	5 (12.5%) [*]	23 (17.0%) ^{**}	0
<i>Aspergillus fumigatus</i>	MI (mean ± SD)	0.9403±0.1493	0.8936±0.0853 ^c	0.8664±0.0753 ^c	0.8976±0.0736 ^c	0.9056±0.1018 ^c	0.9866±0.0963
	Positive reactions (n, %)	5 (14.7%) [*]	5 (17.2%) [*]	3 (9.4%)	5 (12.5%) [*]	18 (13.3%) [*]	0

MI - migration index; ^{a-c}: significantly smaller compared to reference group; ^a p < 0.05, ^b p < 0.01, ^c p < 0.001; ^{*,***,****}: significantly greater compared to reference group; ^{*} p < 0.05, ^{**} p < 0.01, ^{***} p < 0.001.

distinctly lower compared to that noted by Zuskin *et al.* (60.0%) in a similar group of workers [39].

The observed low frequency of positive skin reactions to herbal extracts does not exclude the role of herbal substances in causing allergic or immunotoxic reactions. It is noteworthy that some immunologically active components, e.g. thymol (constituent of thyme), could not be detected in routine skin tests with the use of herbal extracts [38].

An apparently paradoxical result was obtained in sage farmers, of whom 9.4% had positive skin reactions to chamomile extract but a lack of reaction to sage. This could probably be explained by the fact that these farmers, cultivating herbs for a long period (on average 17 years), became allergic to chamomile and then began to cultivate sage instead. As both herbal species belong to different systematic groups, these farmers did not become allergic to sage.

The observed lack of positive precipitin reactions to herbal extracts confirms the results obtained earlier in the precipitation test with other plant extracts [10].

High prevalence of positive precipitin reactions to *Pantoea agglomerans* in all groups of workers exposed to herbs is in accordance with the results obtained by Dutkiewicz *et al.* [12] in the population of herb processing workers and by Skórska *et al.* [34, 35] in grain and flax farmers. Precipitin response of total herb workers to *Alcaligenes faecalis*, another Gram-negative bacterium common in organic dusts, was much lower compared to the results obtained by the above cited authors [12, 34, 35].

The frequency of positive findings in MIF test with all antigens used was high and significantly greater compared to the reference group. Results similar to this test were noted by Skórska *et al.* [35] in flax farmers.

The risk of allergy to microorganisms associated with herb dust seems to be greatest among thyme farmers, who showed in all tests the highest incidence of positive reactions. These results are in accordance with the data reported by Krysińska-Traczyk *et al.* [17] who examined microbiological air samples taken during cleaning of

dried thyme herb and recorded large concentrations of microorganisms, including potentially pathogenic species tested in the present study. It cannot be ruled out that allergy to environmental microbes may contribute to adverse respiratory and skin effects described after exposure to thyme dust, which have been attributed mainly to thymol and other toxic constituents of thyme volatile oil [38]. The limitation of this study is the lack of immunological tests with thyme extract which could not be performed for technical reasons. Such tests, aiming to elucidate the adverse role of the plant and microbial factors present in thyme dust, are in progress.

The results of the present study suggest the important role of *Pantoea agglomerans* (syn. *Erwinia herbicola*, *Enterobacter agglomerans*) as an occupational allergen in herb dust. *P. agglomerans* is an epiphytic bacterium that occurs commonly on the surface of many plants [7, 8, 33]. Large concentrations of these bacteria were found in the air contaminated with dusts from grain, cotton and other plant materials [8, 15, 31, 32]. They have been isolated also from the air contaminated with dust from herbs [11, 17]. *Pantoea agglomerans* produces a potent endotoxin [7, 24, 31, 33] and induces strong immunologic response and inflammatory reaction in the lungs of exposed humans and animals [9, 18, 25, 28, 31]. It was identified as a cause of allergic alveolitis [18, 26] and other respiratory disorders [5, 6] in agricultural workers exposed to grain dust.

The results of this study corroborate with those obtained by Mackiewicz *et al.* [23] who indicated *Pantoea agglomerans* as a possible cause in a case of allergic alveolitis due to exposure to thyme dust. This presumption was based on the positive results of MIF test and inhalation challenge with the antigen of *P. agglomerans*.

Besides significant risk of allergy to *Pantoea agglomerans* in all herb workers, the results of the precipitation and MIF tests showed that thyme farmers are under increased risk of sensitization to *Saccharopolyspora rectivirgula*, *Aspergillus fumigatus*, *Alcaligenes faecalis*

and *Arthrobacter globiformis*, while chamomile and sage farmers are under increased risk of allergy to *Bacillus subtilis* and *S. rectivirgula*. All these microorganisms have been reported as causative agents of allergic alveolitis [16, 19, 26].

CONCLUSIONS

High frequency of positive allergic reactions to environmental microbial antigens in people cultivating or processing herbs suggests a potential role of these factors in the pathogenesis of health disorders caused by exposure to dust from herbs. The risk of sensitization seems to be greatest among thyme farmers, who showed the highest positive response. A particularly high prevalence of positive reactions to *Pantoea agglomerans* among herb workers confirms the significance of this epiphytic Gram-negative bacterium as an occupational allergen.

High response of herb workers to environmental allergens indicates the need for application of appropriate prevention measures. This is important as the number of people occupationally exposed to dust from herbs is increasing.

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