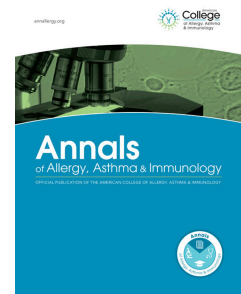


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PII: S1081-1206(20)30007-7

DOI: <https://doi.org/10.1016/j.anai.2020.01.002>

Reference: ANAI 3124

To appear in: *Annals of Allergy, Asthma and Immunology*

Received Date: 15 September 2019

Revised Date: 31 December 2019

Accepted Date: 2 January 2020

Please cite this article as: Aberumand B, Rawls M, Adams DE, Steacy LM, Ellis AK, Possible sources of Bermuda grass sensitization in Timothy grass allergic subjects, *Annals of Allergy, Asthma and Immunology* (2020), doi: <https://doi.org/10.1016/j.anai.2020.01.002>.

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Possible sources of Bermuda grass sensitization in Timothy grass allergic subjects

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Declaration of funding for the research reported in the manuscript: none

Disclosures: AKE has participated in advisory boards for ALK Abello, Circassia Ltd, GlaxoSmithKline, Johnson & Johnson, Merck and Novartis, and has been a speaker for Aralez, AstraZeneca, Boehringer Ingelheim, Meda, Mylan, Merck, Novartis, Pediapharm, Pfizer, and Takeda. Her institution has received research grants from Circassia Ltd, Green Cross

Pharmaceuticals, GlaxoSmithKline, Sanofi, Sun Pharma, Merck, Novartis and Pfizer. AKE is also a consultant to Bayer Inc. BA, MR, LMS and DEA have no conflicts of interest to disclose.

Keywords

Bermuda grass, Timothy grass, pollen cross-reactivity, pollen allergen, immunotherapy, Phleum pratense, Cynodon dactylon, primary sensitization, secondary sensitization

Abbreviations Used

IgE: immunoglobulin E

IgG4: immunoglobulin G4

SPT: skin-prick test

WHO: World Health Organization

IUAC: International Union of Immunological Societies

SLIT: sublingual immunotherapy

SCIT: subcutaneous immunotherapy

Th1: Type 1 T helper

Th2: Type 2 T helper

OAS: oral allergy syndrome

Word Count: 1099

Figures: 2

Allergic rhinitis (AR) is an IgE-mediated disorder that results in nasal inflammation. AR is believed to affect 15-30% of the United States of America (US) population with similar rates observed in Canada [1,2]. Grass pollens represent a major cause of AR [1]. Timothy grass (*Phleum pratense*) from the Pooideae subfamily and Bermuda grass (*Cynodon dactylon*) from the Chloridoideae subfamily are two common grasses that can provoke AR symptoms [3].

Depending on the climate and geographical diversity, the type of grass prevalent in a certain area varies. Regional specificity leads to variability in the exposure of the dominant clinically significant allergen [3]. For instance, temperate grasses such as Timothy are often found in the northern parts of North America and Europe. In contrast, subtropical grasses, such as Bermuda, are prevalent in the warmer climates of Africa, Asia, Australia, Central and South America, and the Southern US [4-7] (**Figure 1**).

A low degree of cross-reactivity has been reported between Timothy and Bermuda grass. Low cross-reactivity has been attributed to the differences in plant systematics. Different species of grasses can have similar physiological, chemical and immunological properties due to overlapping homologues or allergenic components causing the allergenic similarity [8]. Currently 150 allergens have been identified from 52 grass species and classified into 13 grass pollen allergen groups via the World Health Organization (WHO) and the International Union of Immunological Societies (IUAC) accepted nomenclature [5].

Primary sensitization occurs when an individual comes in contact with the allergens contained in the pollen leading to the production of allergen specific IgE antibodies and the development of allergic respiratory diseases. Secondary sensitization, which is the basis of cross-reactivity, occurs when an individual has not come into contact with the allergen in question but exhibits the symptoms of allergic disease similar to those with primary sensitization [9].

Consequently, the distribution of grasses affects the sensitization and antibody cross-reactivity profiles of individuals [7].

In the present study, we investigated a group of participants who had evidence of dual positive skin-prick test (SPT) to Bermuda and Timothy grass by evaluating potential routes of sensitization through residence history.

This study was granted ethical clearance by the local Research Ethics Board. We collected data from AR study participants from past studies from the Allergy Research Unit in Kingston, Canada. Participants were informed via telephone about a survey inquiring about residence history. Verbal consent was obtained prior to initiating the questionnaire. Inclusion criteria for the survey were: age ≥ 18 , residence in Ontario, and evidence of sensitization via SPT to both Timothy and Bermuda grass. A positive SPT was defined as a wheal size of ≥ 3 mm than the negative control.

The total questionnaire took three to five minutes to complete and consisted of the questions outlined in **eFigure 1**. The number of questions varied according to the answer provided in the preceding question. Data was collected in print form and inputted into Microsoft Excel on an encrypted network.

100 participants out of 138 that met the inclusion criteria consented and completed the questionnaire. 69% were female and 31% were male (ages between 19-63). 15% of the participants reported living outside of Canada for more than one year. Only 6% of the participants indicated having ever lived in the US for some period of time. The amount of time lived in the US varied from six months to 18 years (**Figure 1**). Internationally, 22% of the participants had lived in countries excluding Canada and the US, for anywhere between three months to five years (**Figure 1**). Bermuda grass wheal size for these participants ranged from 3 -

13 mm (mean = 7 mm), whereas the Timothy grass wheal ranged from 7 - 20 mm (mean = 12 mm).

Grass pollen remains a significant cause of allergic respiratory disease worldwide [3]. Among them, Timothy and Bermuda grass are found in varying geographical locations due to the required climate conditions. In Ontario and the majority of Canada, Timothy grass dominates. Conversely, in warmer climates such as Southern US, Bermuda grass is more frequently found. Despite the regional specificity in pollen allergies, a high rate of sensitization to both types of grass were noted in residents of Ontario. We found that a resounding number of participants sensitized to both Timothy and Bermuda grass had no residence history indicative of exposure to Bermuda grass. This opens the possibility that the principle route of sensitization is through a secondary process, whereby in vivo cross-reactivity plays a more significant role than previously believed. A similar phenomenon has been observed in participants from Texas where there is minimal exposure to Timothy grass. In comparison to their counterparts who had been naturally exposed to Timothy grass, participants never exposed displayed the same symptomatic response to Timothy grass pollen when challenged in an allergen challenge chamber [6].

Previous in vitro studies have suggested that Chloridoid and Pooid pollen do not cross-react with each other [10]. However, this remains controversial as recent in vitro studies have reported a weak cross-reactivity between Bermuda grass pollen and Timothy grass pollen likely due to the unique group allergens present in each type of grass pollen [4]. Sequence homology and structural comparison between Phl p 1, a major Timothy grass pollen allergen and Cyn d 1, a major Bermuda grass pollen allergen, indicated a small area of conserved amino acid sequence surface area between these two species [7]. Immunoblotting using serum samples from ten Bermuda grass pollen allergic patients have allowed for better characterization of the prevalent

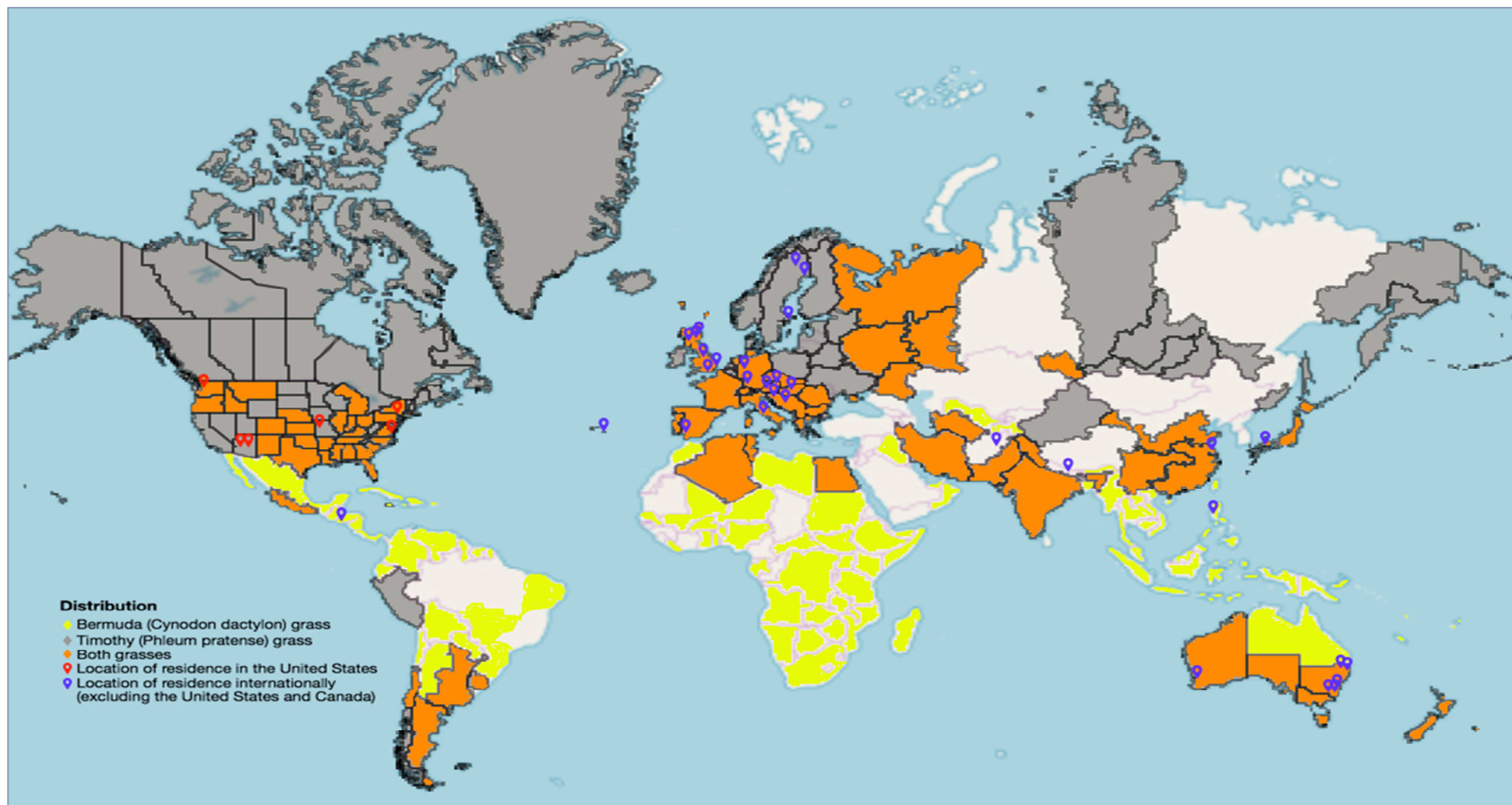
IgE-reactive proteins in Bermuda grass and have identified two Ph1 p 11 homologues with N-terminal sequences 80-90% identical to that of the allergen Ph1 p 11 found in Timothy grass [11]. Our study adds to the growing body of literature that indicates there is more substantial cross-reactivity observed in vivo than traditionally believed [6]. These observations could have important implications in the treatment of grass-pollen allergies especially in an era of climate change, globalization and the expansion of grasses from their original habitation worldwide.

Our study is not without limitations. Firstly, the data reflects only one center in Canada with a cohort of 100 participants. There is also a lack of data regarding the Bermuda grass pollen levels in Ontario. Despite this, our study suggests that prior exposure to common regions where Bermuda grass flourishes is not a prerequisite to develop a sensitization. This may suggest a stronger degree of cross-reactivity between Timothy and Bermuda grass than previously believed. Further studies are needed to uncover the exact mechanism of cross-reactivity between the two grasses and to determine the most prevalent sensitization profiles to create allergen combinations for use in immunotherapy.

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Figure 1: Distribution of Bermuda grass and Timothy grass along with participants' location of residence worldwide excluding Canada



Adapted from Simon, B.K et al. [12]

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eFigure 1: Grass Pollen Sensitization Travel and Residence Questionnaire