Chapter 12
Allergenicity to *Cannabis sativa* L. and Methods to Assess Personal Exposure

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Abstract *Cannabis sativa*, commonly referred to as marijuana, is popularly recognized as a medicinal and recreational drug. Although the legal status of the plant and its derivatives has been debated in numerous social and legal forums, very little is known about the immunological effects following personal and more recently, occupational exposure. Current studies have shown that direct handling and consumption of *C. sativa* and its derivatives can elicit allergic reactions and in very rare cases, life-threatening anaphylaxis. Initially, Δ^9-THC and cannabinol were suggested to be the potential allergic sensitizers; however, recent reports have demonstrated that allergic reactions to *C. sativa* may be driven by type I hypersensitivity mechanisms. In this chapter, we will examine the scenarios and routes of exposure to *C. sativa* that may result in allergic sensitization and provide insights into the key allergic determinants. Finally, the methodological challenges associated with studying the plant and the biotechnological advances in exposure assessment will be additionally discussed.

12.1 Introduction

Cannabis sativa (hemp) is an annual herbaceous plant that belongs to the order, *Rosales* and is placed within the Cannabaceae family. Other common genera found in this family include *Celtis* (hackberry) and *Humulus* (hop). *C. sativa* is widely

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distributed throughout the world, thrives in diverse environmental conditions, and is an intrinsic part of many cultural practices. *C. sativa* is primarily cultivated for industrial hemp that is used for the manufacture of textiles, yarn, fiber, insulation, and cordage. *C. sativa* is also cultivated and consumed popularly as marijuana; a medicinal and recreational drug. *C. sativa* is also used medicinally as an antiemetic drug for the treatment of anorexia, nausea, and severe pain (Asbridge et al. 2012; Hall and Degenhardt 2009; Hurlimann et al. 2012; Lamarine 2012; NIDA 2010; Shapiro and Buckley-Hunter 2010). Much work has focused on tetrahydrocannabinol (Δ^9-THC); a potent psychoactive drug present in the leaves (4% dry weight) and buds (20–30% dry weight) of *C. sativa*. The buds are covered with tiny glandular crystals called trichomes (50–100 μm in size) that contain high concentrations of Δ^9-THC, cannabinoids and terpenes. The trichomes are predominantly present on the buds but can also extend to the surrounding small leaves. Two species, *C. sativa* and *C. indica,* contain greater concentrations of Δ^9-THC, and are more extensively cultivated compared to *C. ruderalis*; which is a low- Δ^9-THC yielding variety.

Adverse effects of *Cannabis* consumptions have been extensively documented in the literature (Hall and Degenhardt 2009; Volkow et al. 2014). More recently, brief reports of allergic sensitization from handling and inhalation of *C. sativa* and its products have emerged (Aldington et al. 2007; Hall and Degenhardt 2009; Howden and Naughton 2011; Lee and Hancox 2011; Reid et al. 2010; Tashkin et al. 1987; Tashkin 2005; Van Hoozen and Cross 1997). To date, a little over 100 articles have been published in the peer-reviewed literature demonstrating that exposure to *C. sativa* can cause allergic reactions and in rare situations culminate in life-threatening anaphylaxis.

12.2 Scenarios of *C. sativa* Exposures

Allergic reactions to *C. sativa* have been predominantly characterized in the context of personal exposures. In the United States, although *Cannabis* is currently classified as a Schedule I substance under the federal Controlled Substances Act of 1970, the regulations are increasingly being relaxed in many of the constituent states. This has contributed to an increase in the workforce associated with *Cannabis* cultivation and processing, highlighting the need for a greater understanding of the potential occupational health impacts. Furthermore, the plant has been known to grow as a part of natural flora in many regions of the world, including the United States, and very little is known of allergic sensitization as a result of environmental exposure to the plant. In this section we will examine the possible scenarios under which exposure to *Cannabis* has been reported to occur.

12.2.1 Personal Exposure

As noted earlier, cultivation and use of marijuana is restricted in many countries and in certain states within the U.S where regulation of cultivation, possession and use of marijuana varies by law in each state (Cerda et al. 2012). However, in the U.S. alone, approximately 10% of *Cannabis* users consume the plant on a daily basis (UNODC 2008) and the recreational use of *Cannabis* is gradually increasing (Adlaf et al. 2005; Degenhardt and Hall 2012; Hall and Degenhardt 2009; Nichols et al. 2014; NIDA 2010; Webb et al. 1996). Globally, according to the estimates reported by the United Nations Office of Drugs and Crime (UNODC), approximately 2.5–5% of global citizens illicitly use *Cannabis* (UNODC 2011).

Cannabis is consumed predominantly by smoking dried preparations of leaves and flowers, but other preparations include ingestion of cakes, slabs or teas (Tessmer et al. 2012), and in some cases, injections through an intravenous route (Hyun et al. 1978; Mims and Lee 1977; Perez 2000). Hashish, a resinous derivative of *C. sativa* exudate (Brown 1998; Herzinger et al. 2011) and hashish oil (Ashton 2001) are also commonly consumed. Hempseed contains a high protein content (25%) and is utilized in various nutritional products, health food supplements, and is also traded as bird feed and fish bait (Callaway 2004; Karus 2004). Recent reports have also emphasized the dermal reactions in individuals directly handling the plant and its derivatives.

Frequent exposure to *Cannabis* may precipitate into a variety of adverse health effects that range from psychosocial effects (Ashton 2001; Hollister 1986), to dermal effects, to respiratory distress including bronchitis, emphysema and allergy (Ashton 2001; Henderson et al. 1972; Larramendi et al. 2013). Very rarely, allergic reaction to *Cannabis* smoke has also been reported from passive exposure (Ebo et al. 2013). However, sensitization to *Cannabis* is not only restricted to the inhalation of marijuana smoke. Many studies have commonly reported dermal reactions such as urticaria that manifests as an erythematous rash in individuals in direct contact of *Cannabis* or its derivatives (Table 12.1) (Basharat et al. 2011; Perez-Bustamante et al. 2007) (Ozyurt et al. 2014). Overall, it appears that allergic reactions are more common when handling the plant as opposed to smoking it.

In rare cases, anaphylactic reactions have been reported in atopic subjects following smoking *Cannabis* or ingesting marijuana tea (Liskow et al. 1971; Tessmer et al. 2012). Typically, individuals that are sensitized to *C. sativa* have been determined to be atopic and previously sensitized to other allergens such as pollen, dander, dust mites, or fungi. Very few studies have reported sensitization to *C. sativa* in non-atopic individuals (Rojas Perez-Ezquerra et al. 2014). Table 12.1 provides a brief summary of allergic reactions to *Cannabis* exposure that are presented in the peer reviewed literature.

The impact of long term versus short term marijuana exposure on respiratory symptoms remains largely unclear (Tetrault et al. 2007). Some studies have reported that marijuana smoking may lead to airway inflammation and obstruction along with short-term acute bronchospasm and possible long-term emphysema with

Table 12.1 Personal and environmental allergy cases

Location	# patients in study	Route of exposure	Clinical presentation	Allergens (molecular weights)	Reference
Madrid, Spain	1	Smoking and contact	Papular lesions, itching, generalized erythema and palpebral angioedema	10, 26, 38, 69	(Perez-Bustamante et al. 2007)
St. Louis, MO (USA)	1	Smoking	Anaphylaxis, nasal and pharyngeal pruritus, lacrimation, nasal congestion, dyspnea and wheezing	N/A	(Liskow et al. 1971)
Basel, Switzerland	1	Smoking	Itching, rhinoconjunctivitis	N/A	(Stockli and Bircher 2007)
Toronto, Canada	7	Smoking and contact	Rhinitis, conjunctivitis, periorbital angioedema and dermatitis. One patient with anaphylaxis resulting in GI cramping and vomiting.	N/A	(Basharat et al. 2011)
Bilbao, Madrid and Pamplona, Spain	1	Smoking and contact	Urticaria, rhinorrhea, palpebral edema, itching	9, 14, 35 and 100	(Gamboa et al. 2007)
Madrid, Spain	1	Smoking and contact	Itching, rhinorrhea, pharyngeal pruritus	N/A	(Anibarro and Fontella 1996)
Miami, FL (USA)	1	Smoking mold contaminated marijuana	Fever, wheezing	N/A	(Llamas et al. 1978)

(continued)

Table 12.1 (continued)

Location	# patients in study	Route of exposure	Clinical presentation	Allergens (molecular weights)	Reference
Milwaukee, WI (USA)	28	Smoking mold contaminated marijuana	Mostly asymptomatic, some with bronchospasm	N/A	(Kagen et al. 1982)
Valladolid, Spain	140 total with 53.2% patients positive for *Cannabis* skin prick test and 34.3% patients positive for specific IgE	General allergic patients and drug-dependent users	Asthma, urticaria, rhinitis and anaphylaxis in some cases	N/A	(Armentia et al. 2011)
Benidorm, Tres Cantos and Orihuela, Spain	32 patients atopic to tomato including 10 with allergic reactions to *Cannabis* exposure	Tomato-sensitized patients with contact or inhalation exposure to *Cannabis*	Urticaria, rhinoconjunctivitis, itching and in some cases asthma-like symptoms and palpebral angioedema	9–10, 14, 30–35, 45	(de Larramendi et al. 2007,2008)
Multi-center study, Spain	44 patients tested positive for *C. sativa*	Smoking and handling of *C. sativa*	Broad respiratory and cutaneous symptoms with sporadic cases of anaphylaxis	10 and 14 kDa (LTP) and 38 kDa (thaumatin-like protein)	(Larramendi et al. 2013)
Toronto, Canada	17	Smoking and contact exposure. One patient consumed marijuana tea	Rhinitis, conjunctivitis, sinusitis, wheezing, contact urticaria with periorbital angioedema and abdominal cramping in few cases	Major immunoreactive bands at 50 and 23 identified as RuBisCO and OEP2 respectively. Other minor allergens ranged from 10 to 100 kDa.	(Nayak et al. 2013; Tessmer et al. 2012)
Bakersfield, CA (USA)	1	Intravenous personal exposure	Facial edema, pruritus, wheezing and dyspnea	N/A	(Perez 2000)

(continued)

Table 12.1 (continued)

Location	# patients in study	Route of exposure	Clinical presentation	Allergens (molecular weights)	Reference
Munich, Germany and Valladolid, Spain	16 (8 Spanish and 8 German)	All 8 Spanish patients were identified as drug-dependent users of *Cannabis*. *Cannabis* use was reported unknown among German patients	Asthma, conjunctivitis, rhinitis and urticaria were common manifestations. In a rare case anaphylaxis was also noted.	IgE reactivity specifically tested to Pru p 3 (LTP)	(Rihs et al. 2014)
Antwerp, Belgium	21 patients reporting oral allergy syndrome (OAS) or food-related allergies, including 12 patients with respiratory and cutaneous reactions to *C. sativa*	All 12 patients were identified to have exposure to marijuana or hashish by smoking or cutaneous transmission	Rhinitis, conjunctivitis, asthma and contact urticaria and itching	N/A	(Ebo et al. 2013)
Fukuoka, Japan	1	Uncharacterized exposure	Uncharacterized clinical observations	10, 14, 45, 60 and 68	(Tanaka et al. 1998)
Izmir, Turkey	1	Smoking exposure	Erythematous, vesiculobullous, scaly rash	N/A	(Ozyurt et al. 2014)
Tucson, AZ (USA)	63	Environmental exposure to *C. sativa* pollen	Allergic rhinitis, asthma, urticaria and atopic dermatitis	N/A	(Freeman 1983)
Papillion, NE (USA)	127 total with 78 patients with SPT determined towards *C. sativa* extract	Environmental exposure to *C. sativa* pollen	Rhinitis and asthma/asthma-like symptoms	N/A	(Stokes et al. 2000)
Lucknow, India	48 total with 7 patients with SPT determined towards *C. sativa* pollen	Possibly environmental exposure to *C. sativa* pollen	Nasobronchial allergy	N/A	(Prasad et al. 2009)

a strong correlation between bronchodilation and some of the clinical symptoms (Tashkin 2005; Wolff and O'Donnell 2004), while others have presented contradictory findings that *Cannabis* smoking does not appear to augment the risk of developing emphysema.

The dearth of literature pertaining to the consumption of this plant is likely due to the legal and social implications associated with its cultivation, handling and possession. It is possible that many individuals may continue to consume the plant and its by-products and not report allergic reactions or seek medical treatment for fear of criminal prosecution. The reported incidence of adverse reactions such as anaphylaxis following *Cannabis* consumption further highlight the need of increased awareness in the general population as well as the medical community. This could help provide prompt therapeutic interventions and resolution of the allergic symptoms.

12.2.2 Occupational Hemp Exposures

C. sativa has been an essential industrial commodity throughout human history, particularly for the manufacturing and textile industries. Soft hemp derived from the plant is rich in fiber and has been used in the manufacturing of cordage (ropes), rugs, paper, clothing, biodegradable plastics, and even forms an essential component of some construction and insulation materials. However, advances in the field of material sciences and restrictions on cultivation of *Cannabis* in some regions of the world have limited its application in the modern commercial processes, with only a modest number of hemp industries currently in operation.

Occupational exposure to *C. sativa* was initially described among hemp workers in the early 18th century (Zuskin et al. 1990). Byssinosis (occupational brown lung disease) has been reported in hemp workers following the inhalation of organic dust. In European longitudinal cohort studies, exposure to retted soft hemp has been shown to be a major risk factor for allergic sensitization (Barbero and Flores 1967; Bouhuys and Zuskin 1979; Fishwick et al. 2001; Smith et al. 1969; Valic et al. 1968; Zuskin et al. 1990, 1994). Hemp processors involved in direct handling of the plant are particularly at risk due to ongoing chronic exposures. These workers commonly present symptoms characterized by reduced respiratory function (Barbero and Flores 1967; Valic and Zuskin 1971; Zuskin et al. 1994). Workers demonstrated significantly higher prevalence of chronic symptoms of cough and phlegm as well as shortness of breath and chest tightness when compared to control subjects (Zuskin et al. 1990, 1994). Upon skin prick test (SPT), workers also demonstrated a positive reaction to extracts derived from different origins within the processing operation (Chen 1986; Zuskin et al. 1992). Occupational tasks such as handling raw hemp appeared to contribute to a higher prevalence of SPT positivity with a strong correlation to respiratory illness. However, other studies have found insufficient correlation between allergic sensitization to *C. sativa* and the respiratory health of individual workers (Fishwick et al. 2001).

Although *C. sativa* cultivation for industrial purposes has diminished in modern times, given the economic benefits of cultivating *C. sativa* for medicinal and recreational uses, more than 30 countries grow this plant for distribution (Johnson 2012). As a result, a substantial population of workers are routinely engaged in the cultivation, handling, processing and manufacturing of *C. sativa*. Furthermore, increasing legalization of the plant for its medicinal and recreational use has generated an emerging workforce. In the United States, the *Cannabis* industry is in its emerging stages of growth and has minimal standardized industrial work practices. This is a concern for an increasing number of workers who handle the plant daily and are at risk of developing a wide range of health issues. Many of the processes involved in cultivation of C. *sativa* involve direct handling of the plant. More specifically, workers involved in the role of 'trimming' are at an increased risk of developing allergic reactions to the plant due to prolonged direct contact with the plant. The 'trimming' process involves removing the outer fan leaves and small leaves and conducting precision adjustments to the *Cannabis* 'bud'. Organic dust generated during various stages of manufacturing processes is also likely to exacerbate allergic reactions.

Direct handling of *Cannabis* has been demonstrated to drive urticaria in forensic and law enforecement officers. (Herzinger et al. 2011; Lindemayr and Jager 1980; Majmudar et al. 2006; Mayoral et al. 2008; Williams et al. 2008). Collectively, these reports emphasize that occupational exposure to *Cannabis* can stimulate allergic reactions in workers who are in close proximity to or handling the plant. Further, both inhalation as well as dermal exposure appears to be relevant in these scenarios. Although the underlying immunological mechanisms remain uncharacterized, a strong correlation exists between serological abnormalities and the associated respiratory and dermal symptoms in workers that handle *C. sativa*.

12.2.3 Environmental Exposures

C. sativa is an anemophilous plant that produces large quantities of pollen. Morphologically, *C. sativa* pollen are trizonoporate, (sometimes tretrazonoporate), and approximately 30 μm in size (Aboulaich et al. 2013). Large quantities of pollen are produced by each inflorescence ($\sim$350,000) and can be disseminated over long distances (Aboulaich et al. 2013). For example, *Cannabis* pollen derived from African geographic locales has been traced as far away as Europe, more specifically in Spain (Cabezudo et al. 1997). *C. sativa* is widespread in many regions of the world and environmental exposures to *C. sativa* pollen may be more prevalent than reported and may contribute towards the development of allergic sensitization to *Cannabis* in these regions.

Very few studies have assessed the influence of *C. sativa* pollen exposure to elicit rhinitis and asthma in the U.S. (Freeman 1983; Stokes et al. 2000). In the southwest region of the U.S., one study reported that people may become sensitized to *C. sativa* pollen (Freeman 1983). Another study, conducted in the Midwest

region of the U.S. reported a strong correlation between skin test reactivity to *C. sativa* pollen, respiratory symptoms, and *C. sativa* pollen counts determined during different months (Stokes et al. 2000). In Europe, the allergenic potential of *C. sativa* pollen has also been reported. In one study, rhinitis and asthma symptoms were attributed to environmental exposure to *C. sativa* and other related plants of the Cannabaceae family, such as, *Celtis* (hackberry) and *Humulus* (hops) among others (Torre et al. 2007). At present, environmental sensitization to *Cannabis* does not appear to be a major concern. However, increased cultivation of *Cannabis* may increase exposure in these regions.

12.3 Allergens of *Cannabis sativa* and Cross-Reactivity

There is a growing body of evidence that *C. sativa* allergens are the cause of type I hypersensitivity reactions. Molecular analyses have provided significant insights into the potential protein allergens of *C. sativa* (de Larramendi et al. 2008; Gamboa et al. 2007; Larramendi et al. 2013; Mayoral et al. 2008; Nayak et al. 2013; Rojas Perez-Ezquerra et al. 2014; Tanaka et al. 1998). High molecular weight allergens ranging from 10–100 kDa have been reported in some of these studies and are collectively presented in Tables 12.1 and 12.2.

Can s 3, a lipid transfer protein (LTP) is the only *C. sativa* allergen that is currently recognized by the International Union of Immunological Societies (IUIS) (Rihs et al. 2014). Multiple studies have reported LTP as a major allergen of *Cannabis* particularly in Europe (de Larramendi et al. 2008; Gamboa et al. 2007; Larramendi et al. 2013; Perez-Bustamante et al. 2007; Rojas Perez-Ezquerra et al. 2014; Tanaka et al. 1998). LTPs are found in all plants and are thought to play a role in plant defense against pathogens and stress by the transfer of lipids for synthesis of the protective cuticle of the plant. Many LTPs are considered allergens and have been reported as major sensitizers in oral allergy syndrome to fruit (peach, cherry and apple), and also as inhalational sensitizers (Enrique et al. 2005; Palacin et al. 2007). LTPs are expressed as a polypeptide approximately 10–14 kDa in size including a signal peptide, which is cleaved, thus forming a mature protein of approximately 9 kDa in molecular size (Salcedo et al. 2004). It shares significant sequence homology with other plant derived LTP allergens (Fig. 12.1). LTPs are highly thermostable and resistant to proteolytic degradation making them a concern for systemic and more severe reactions (anaphylaxis) (Breiteneder and Mills 2005). Recombinant LTP from cannabis (rCan s 3) has been expressed with maltose-binding protein (MBP) as a purification tag ($\sim$52 kDa fusion protein) (Rihs et al. 2014).

LTPs are increasingly being identified as pan-allergens that can drive severe systemic reactions (Breiteneder and Mills 2005). Mechanistically, the highly conserved sequences contribute to cross-reactions with other plant sources containing homologous proteins. Collectively, this is referred to as sensitization to non-specific LTPs (nsLTPs). Some studies have described patient IgE cross-reactivity between

Table 12.2 Occupational *Cannabis* allergy cases

Location	Number of patients in study	Route of exposure	Clinical presentation	Allergens (molecular weights)	Reference
Munich, Germany	2	Long term occupational contact exposure	Nasal congestion, rhinitis, sneezing	N/A	(Herzinger et al. 2011)
Leeds and York, United Kingdom	1	Long term occupational contact exposure	Urticaria	N/A	(Williams et al. 2008)
Solihull, United Kingdom	1	Long term occupational contact exposure	Rhinitis, headache, urticaria	N/A	(Majmudar et al. 2006)
Vienna, Austria	1	Long term occupational contact exposure	Urticaria	N/A	(Lindemayr and Jager 1980)
Madrid, Spain	1	Occupational exposure through inhalation of *C. sativa* seed fragments	Rhinorrhea, chest tightness, dyspnea, wheezing	N/A	(Vidal et al. 1991)
Zagreb, Croatia and New York City, NY (USA)	111 in the first study and 66 in the follow-up study	Occupational exposure in soft hemp processing mill	Chronic cough, phlegm, dyspnea, etc.	N/A	(Zuskin et al. 1990, 1994)
Sheffield and Norfolk, United Kingdom	11	Occupational exposure in hemp processing mill	Chest tightness and wheezing in some patients	N/A	(Fishwick et al. 2001)
Granada and Madrid, Spain	1	Occupational exposure at a research laboratory	Perennial rhinoconjunctivitis	37 and two bands at ~70–80	(Mayoral et al. 2008)
Madrid, Spain	1	Occupational exposure from handling plant leaves during cultivation	Wheals and pruritus	12 and 14	(Rojas Perez-Ezquerra et al. 2014)

Fig. 12.1 Sequence alignment of partial sequences of lipid transfer protein (LTP) from *Cannabis sativa* (Can s 3), *Juglans regia* or walnut (Jug r 3), *Prunus persica* or peach (Pru p 3), *Arachis hypogaea* (Ara h 9), *Corylus avellana* or hazelnut (Cor a 8), *Artemisia vulgaris* or mugwort (Art v 3)

purified native *C. sativa* LTP (Can s 3) and the homologue from peach (Pru p 3) (Gamboa et al. 2007). A recent study in Europe noted that *C. sativa*-atopic subjects with accompanying food allergy demonstrated higher IgE titers and exhibited more severe allergic reactions compared to *C. sativa*-non-atopic group (Ebo et al. 2013). Patients showed positive immunoreactivity to nsLTPs from various plants including peach (Pru p 3), plane tree (Pla a 3), walnut (Jug r 3), hazelnut (Cor a 8), peanut (Ara h 9) and mugwort (Art v3). Cross-reactivity in a smaller group of patients was observed towards nsLTP from wheat (Tri a 14) and olive (Ole e 7). A large number of *C. sativa* sensitized subjects also reacted to the major Birch allergen (Bet v 1) and grass allergen (Phl p 1), members of the pathogen-related (PR-10) protein family. Although, various pollen allergens are thought to drive allergic sensitization to various foods, it is becoming widely accepted that sensitization to nsLTPs may be the primary mechanism.

Although Can s 3 is the best characterized allergen of *C. sativa*, other potential allergens have been reported in the literature. A thaumatin-like protein has also been identified as a potential *C. sativa* allergen (Larramendi et al. 2013). In a study conducted in Spain, 3 additional proteins were identified as major allergens along with LTP; profilin (14 subjects), peptinesterases (31 subjects) and polygalactouranase (31 subjects) (de Larramendi et al. 2008). These proteins are noted food allergens in Europe, especially in Mediterranean countries (Ibarrola et al. 2004; Pastorello et al. 2002; Swoboda et al. 2004). In a study by Nayak et al., in North America, IgE binding protein profiles of serum derived from sensitized subjects was assessed in extracts from various parts of the *C. sativa* plant (Nayak et al. 2013). Using sera from *C. sativa*-sensitized patients, Nayak et al., observed comparable IgE binding profiles in extracts from leaves, buds and flowers. Using 2D electrophoresis and proteomic analysis, a 50 kDa ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO) and a 23 kDa protein, oxygen-evolving enhancer protein (OEP2) were identified as common allergens. OEP2 is a relatively unknown allergen, while RuBisCO is a photosynthetic enzyme and one of the most common

proteins in nature with very few studies reporting it to be an allergen (Ahrens et al. 2014; Foti et al. 2012; Hoff et al. 2007). RuBisCO is very susceptible to rapid degradation by gastric enzymes and is not commonly reported as a food allergen (Astwood et al. 1996; Fu et al. 2002). However, parenteral exposure by inhalation or cutaneous administration, may stimulate development of IgE responses to RuBisCO (Bowman and Selgrade 2008; de Lacoste de Laval et al. 2006). Interestingly, in this North American-based study, LTP was not identified as a potential allergen. Although 2 patients demonstrated IgE immunoreactivity at ~10 kDa, LTP was not confirmed by proteomic analysis. While nsLTP sensitization has been reported in the Mediterranean region, similar sensitization is rarely reported among North American cohorts. Collectively, these observations highlight a potential geographical bias for sensitization, although additional studies focused on providing component-based resolution of allergic sensitization are required.

Although sensitization to nsLTPs is a problem, the broad nature of cross-reactivity is becoming an increasing concern. A significant proportion of individuals sensitized to tomatoes developed reactions following handling or inhalation of *C. sativa* (de Larramendi et al. 2007, 2008). On further evaluation, these individuals also demonstrated a positive SPT and specific IgE reactivity towards a soluble *C. sativa* leaf extract. A large number of patients also showed a positive SPT towards the resinous derivative or 'hashish' prepared from *C. sativa* bud-associated glands called trichomes that are rich in THC. In the same study, a group of individuals with prior sensitization to tomato but no known sensitization or exposure to *C. sativa*, were also positive to *C. sativa* by SPT and demonstrated specific IgE in ELISA with *C. sativa* leaf extract. Some patients also exhibited cross-reactivity to *Prunus persica* (peach), *Artemisia vulgaris* (mugwort) and a small number to *Platanus hybrida* (plane tree hybrid). In the southwestern region of the U.S., some patients with allergic symptoms, were SPT positive towards *C. sativa* pollen extract along with pollen from other closely related Rosid plants including hops (*Humulus lupulus*), mulberry, and elm (Freeman 1983). In a study performed in Valladolid, Spain, researchers elaborately described the IgE cross-reactivity and sensitization to *Cannabis* among drug-dependent patients and allergic patients (Armentia et al. 2011). In this study, patients sensitized to *Nicotiana tabacum* (tobacco) and *Solanum lycopersicum* (tomato) were determined to be at a greater risk of being sensitized to *Cannabis* compared to patients sensitized to *Hevea brasiliensis* (latex) and Gramineae pollen. Fifty three percent of patients in the study were identified to have a positive SPT reaction to *Cannabis* extract, while only 34% were determined to have specific IgE to *Cannabis*. This difference could be attributed to the cross-reactive IgE in patients towards tomato, tobacco or latex. More recently, one patient sensitized to Can s 3 was reported to show cross-reactivity to Hev b 12 (latex LTP) (Faber et al. 2015).

Elsewhere, one individual determined to be primarily sensitized to *C. sativa*, at a later point exhibited serious allergic reaction on consumption of tomatoes and pepper (Gamboa et al. 2007). This particular patient did not present sensitization to any foods on previous examination. The patient reported collective symptoms of anaphylaxis to tomato consumption and exhibited contact urticaria to various other

foods especially peach with specific IgE reactivity to the nsLTP (Pru p 3). While previous studies in southern Europe have linked *Cannabis*-sensitization to allergic reactions on consumption of tomato and peach (de Larramendi et al. 2007, 2008); Ebo et al. found that food allergy was predominantly associated with banana, tomato and grape (Ebo et al. 2013).

Cannabis-sensitized individuals are frequently atopic (Armentia et al. 2011; de Larramendi et al. 2008; Ebo et al. 2013; Vidal et al. 1991). In one study, in a patient undergoing immunotherapy for *Dermatophagoides pteronyssinus* (house dust mite) allergy; a type 1 response was reported following the inhalation of *C. sativa* (Vidal et al. 1991). Similarly, patients with positive SPT to *Cannabis*, demonstrated varying SPT reactivity to various other allergen sources including insects, animal dander, dust, ragweed, birch pollen etc. (Prasad et al. 2009; Shivpuri 1980; Stockli and Bircher 2007; Stokes et al. 2000).

In addition to cross-reactivity, sensitization to fungi was reported from smoking mold-contaminated marijuana (Chusid et al. 1975; Kagen et al. 1981, 1982; Kurup et al. 1983; Llamas et al. 1978; Llewellyn and O'Rear 1977). In these studies, fungi were isolated from marijuana that was stored in moist environments. Marijuana cigarettes obtained for mycological analysis demonstrated fungal growth on culture (Kagen et al. 1982; Kurup et al. 1983). *Aspergillus* species, including *A. niger*, *A. flavus* and *A. fumigatus* were frequently isolated along with other fungal contaminants and thermophilic actinomycetes. Based on the information provided in these studies, it is difficult to determine whether sensitization to fungi occurred exclusively from mold-contaminated marijuana, even though the fungal material was isolated. *Cannabis* is highly susceptible to diseases caused by fungal growth. Densely packed buds and flowering tops hold high content of moisture that allows for infestation by molds such as *Botrytis cinerea, Sclerotinia sclerotiorum, Fusarium species* etc. It is possible that patients may also become sensitized to fungi under unrelated conditions and may demonstrate a reaction after inhaling mold-contaminated *Cannabis*. It appears that although contamination of marijuana with fungi may not pose a significant public health burden currently, this problem is a major concern in the context of medical marijuana utilized by mostly immunocompromised patients. Any fungal contamination of medicinal marijuana can be devastating when consumed by this highly susceptible group of patients.

There is growing evidence in the literature on specific interactions of IgE antibodies towards glycosylated motifs on various plant-derived allergens. Using *in silico* methods, only O-glycosylation (n = 3) sites were predicted for Can s 3. Armentia and colleagues showed that 1 out of every 3 *Cannabis*-sensitized patients showed reactivity to cross-reactive carbohydrates (CCDs) and western blot studies conducted by our group have demonstrated possible IgE binding to plant carbohydrates (Armentia et al. 2014; Nayak et al. 2013). Additional studies will be essential in determining the role of cross-reactive carbohydrates in driving allergic sensitization to *Cannabis*.

Cannabis contains a large number of cannabinoids that have been associated with promoting psychoactive effects. Previously it has been suggested that Δ^9-THC may become a potential allergic sensitizer (Liskow et al. 1971). One study reported

allergenicity of 5 cannabinoids, which produced contact dermatitis in experimental animals (Watson et al. 1983). Among the allergic sensitizers were Δ^9-THC, cannabinol, cannabidiol, Δ^8-THC and cannabichromene. The authors suggested that the presence of a free 1′-hydroxyl group was essential for sensitization. In contrast, a recent study showed that THC may alleviate allergic inflammation in mice in a DFNB-mediated allergic contact dermatitis model (Gaffal et al. 2013). To date, the role of THC in allergic sensitization is unclear and further studies are needed.

12.4 Biotechnological Advances in Diagnosis of Allergic Sensitization to *C. sativa*

The clinical evaluation of allergic sensitization to *C. sativa* is a major challenge due to the broad spectrum of symptoms manifested by the patients. Clinical symptoms include itching and urticaria, sore throat, rhinitis and nasal congestion, pharyngitis, wheezing and dyspnea, lacrimation and in very rare cases anaphylaxis (Henderson et al. 1972; Liskow et al. 1971; Perez-Bustamante et al. 2007; Perez 2000; Tessmer et al. 2012) (Tables 12.1 and 12.2). In some cases dermatitis can also be observed (Basharat et al. 2011). Episodes of papular lesions with a general erythema are also common clinical presentations (Perez-Bustamante et al. 2007). Respiratory symptoms are more common in individuals who regularly smoke marijuana (Basharat et al. 2011). Sinusitis has also been reported in certain occupational exposure cases (Zuskin et al. 1990). Additional clinical manifestations include rhinitis and conjunctivitis in most cases with minimal periorbital angioedema (Basharat et al. 2011; Perez-Bustamante et al. 2007).

Current SPT methodologies have primarily used non-standardized extracts derived from the leaves of *C. sativa* (Armentia et al. 2011; Perez-Bustamante et al. 2007), flowers and/or buds (Basharat et al. 2011; Gamboa et al. 2007) or a mixture of leaves and flowers, (Majmudar et al. 2006; Williams et al. 2008). In occupational exposure assessments, extracts have been collected from hemp dust samples from the operating environment (Gupta et al. 1980; Zuskin et al. 1992). *Cannabis* pollen extracts have also been used in occupational exposure cases involving forensic workers (Mayoral et al. 2008). In a specific occupational exposure case involving *C. sativa* seeds, extracts used for SPT, biochemical and immunological analysis were generated from acetone treated seeds (Vidal et al. 1991). *Cannabis* extracts have been prepared using a variety of solvent systems including phosphate buffered saline (de Larramendi et al. 2008; Perez-Bustamante et al. 2007; Rojas Perez-Ezquerra et al. 2014), saline (Anibarro and Fontella 1996; Gamboa et al. 2007; Herzinger et al. 2011), aqueous solution containing carbonate (Vidal et al. 1991) as well as water (Armentia et al. 2014; Tessmer et al. 2012). Elsewhere, sensitization to *C. sativa* has been determined using glycerosaline extracts of pollen in SPT (Freeman 1983; Mayoral et al. 2008). Some studies have developed more detailed methodologies to generate *Cannabis* pollen extracts that involve carbonate

buffer extraction and cellulose column purification (Tanaka et al. 1998). Armentia and colleagues recently described a methodology where fresh *C. sativa* leaves were treated to remove lipids using acetone and cold-milled to preserve other macromolecular contents (Armentia et al. 2011, 2014). The dry material was extracted with Tris in the presence of EDTA and centrifuged at high speed to collect the supernatant. This preparation was then dialyzed against water and used for analysis. The authors reported a high degree of sensitivity and specificity using this extract.

The determination of SPT results has been shown to be variable between studies. A positive SPT to *C. sativa* has either been described as wheals greater than 5 mm and accompanied by a surrounding erythematous flare (Gupta et al. 1980; Vidal et al. 1991), while in other studies a wheal of 5–10 mm has been considered weak reactivity (Stockli and Bircher 2007), whereas other studies have reported wheals greater than 3 mm to be a positive reaction (Mayoral et al. 2008; Zuskin et al. 1992). In one occupational exposure study, the subject was tested with *C. sativa* extracts prepared from leaves, immature flowers as well as fully mature flowers (Williams et al. 2008). The patient demonstrated a smaller wheal of 4 mm in response to extract from leaves and wheals of 13–15 mm were produced to extracts from flowers.

The importance of testing material is further emphasized in a case report presented by Stockli et al. (Stockli and Bircher 2007). The authors reported that one patient who previously tested non-reactive towards *Cannabis* extract from one source, exhibited strong positive reaction when tested with an extract from a different source. This clearly points to the potential variability between testing materials used in diagnosis of allergic sensitization to *C. sativa*.

Although sources of *Cannabis* differ in each study location, it is inferred from the above points that there is tremendous variability in the preparative procedures for generating extracts for testing sensitization predictive of clinical allergic responses. Furthermore, the interpretation of SPT diagnosis is not standardized and relies entirely on investigators' personal judgment. To date, the stability of the allergenic proteins in these extracts over a long storage period has not been comprehensively investigated. Additionally, the safety of applying these rudimentary testing agents is unknown although no serious reactions have been reported. The limited availability of standardized reagents for investigating *C. sativa* sensitization and the broad spectrum of clinical symptoms presented by the patients has prevented thorough and specific evaluation of sensitization (Ebo et al. 2013; Herzinger et al. 2011). Additional research is essential in identifying major allergens and applying recombinant-based methodological advances to assist in clinical evaluation of specific exposure to *Cannabis*.

In recent years, several studies have attempted to improve the available diagnostic methodologies. Some studies have employed radioallergosorbent assay (RAST)-based assays to determine serum IgE titers to *C. sativa* extracts while others have used enzyme-linked immunosorbent assay (ELISA)- based assays (de Larramendi et al. 2008; Ebo et al. 2013; Mayoral et al. 2008; Perez-Bustamante et al. 2007; Tanaka et al. 1998; Zuskin et al. 1992). Tanaka et al., developed an ELISA assay using *Cannabis* pollen for measuring patient IgG and IgE reactivity (Tanaka et al. 1998). Determination of specific IgE using biotinylated *Cannabis* leaf

extract as the solid phase using Phadia ImmunoCAP has shown a high degree of sensitivity and specificity (Armentia et al. 2011). In another study, ~95% of *Cannabis*-sensitized patients tested positive in an array-based method using native purified *Cannabis* LTP (nCan s 3) (Armentia et al. 2014). However, it is possible that other proteins and potential allergens present in *C. sativa* may be co-purified with nCan s 3. Expressing recombinant *Cannabis* allergens for testing would provide better resolution during diagnosis. Recombinant Can s 3 (LTP) has been developed and used in ImmunoCAP-based studies (Rihs et al. 2014). Elsewhere, multiplexed-component-resolved diagnosis (CRD) with native and recombinant LTP proteins from many plant sources has demonstrated the utility of Can s 3 as a useful marker for diagnosis of *Cannabis* allergy (Ebo et al. 2013).

More recently, basophil-activation test (BAT) has been shown to highly discriminate between *Cannabis* sensitized and non-sensitized individuals in individuals with cross-reactive food allergies (Ebo et al. 2013). This technique requires stimulation of peripheral blood cells with an extract from *C. sativa* and assessment of dynamic shifts in expression of CD63 molecule on $CD203c^{+}IgE^{+}$ basophils using flow cytometry. The test requires stimulation of human blood cells with an optimum level of allergen since higher concentrations of the allergen interfere with the accuracy of the test. In future, optimization of *Cannabis* testing reagents may provide considerable reliability to BAT in reporting *Cannabis*-specific allergic reactions.

More recently, our laboratory has established an interest in developing ELISA-based exposure assessment of samples for personal and environmental exposure to *Cannabis*. Theoretically, these assays would also allow for evaluation of *Cannabis* protein burden in environmental samples. Furthermore, our laboratory is developing metagenomic analysis methods to characterize the microbial burden, which may be a potential source of allergenic co-exposures during *Cannabis* cultivation.

Current diagnostics of allergic sensitization to *Cannabis* have many limitations. The choice of plant material, methods of extraction and testing emphasize lack of methodological consistency. The molecular constituents in extract solutions currently remain uncharacterized and non-standardized. Plant components vary in their macromolecular make-up based on the plant features and processing involved. Identification of *Cannabis*-specific allergens and the development of recombinant protein-based diagnostic approaches may be helpful in the future; however reliable markers are currently unavailable.

12.5 Treatment of Allergic Exposure to *Cannabis sativa*

Considering that sensitization to *Cannabis* is a novel phenomenon, very little is known about the available treatment and immunotherapy options. Largely, avoidance of the plant and its by-products appear to help limit allergic episodes (Ozyurt et al. 2014). Some have reported success with immunotherapy using *Cannabis* extracts or

extracts from closely related plants such as *Humulus* (Gupta et al. 1980). In the former case, hyposensitization was performed on hemp workers by intradermal injection with hemp antigens prepared from hemp twine, and hemp fibers. Workers were also treated with an antigenic extract from the thermophilic and thermotolerant microbial constituents associated with the hemp twine. The therapy involved intradermal injections of 50 μl of each antigen extract, twice a week for one year. Following treatment, some workers developed symptoms of mild fever along with inflammation at the site of inoculation. These symptoms persisted for brief intervals and no severe reactions were reported in any worker throughout the course of treatment. Following completion of treatment, the workers showed improved tolerance to these antigens along with improved respiratory vital capacity. All workers showed improvement in symptoms of cough, chest tightness, sneezing, nasal obstruction etc. In another study extract from *Humulus* (hop) was used to develop hyposensitization therapy to *Cannabis* with some success (Lindemayr and Jager 1980).

In non-occupational settings, clinical representations appear to be effectively controlled with antihistamines (Perez-Bustamante et al. 2007; Stockli and Bircher 2007). Treatment with epinephrine, diphenhydramine and methylprednisolone were shown to alleviate the side effects resulting from intravenous administration of marijuana (Perez 2000). Topical steroids can provide temporary relief from dermal symptoms, but remissions and exacerbations have been commonly noted (Ozyurt et al. 2014).

There is a dearth of information on immunotherapy and treatment of exposures to *C. sativa*. The various routes of exposure and the dynamic nature of sensitization continue to be a challenge in treating *Cannabis*-related allergic disease.

12.6 Additional Comments

Cannabis allergic sensitization is a complex condition, which is influenced by the route of exposure, the variety of manifested clinical symptoms and the role of atopy and IgE-driven pathological mechanisms. It is evident that chronic exposure or direct contact with the plant by-products as a result of recreational or occupational exposure could possibly lead to allergic sensitization. However, standardized diagnostic methodologies need improvements. An increasing number of individuals are gaining access to marijuana for recreational or medical purposes, yet the scientific understanding of the plant components and their ability to exacerbate respiratory and dermal reactions is inadequate. As with allergic reactions to other drugs such as penicillin, information on *C. sativa* sensitization may be important in the context of medicinal use of the plant. Extensive research is vital towards gaining deeper understanding of the immunological mechanisms driving the clinical manifestations.

A large proportion of available literature on occupational exposure to *Cannabis* is from the past millennium. The information available to us does not take into account the peculiarities of modern day work practices and the unique challenges posed by these occupational environments.

Some recent literature provides information on cross-reactivity that may influence sensitization; however, most allergens remain uncharacterized. Cross-sensitization may also depend on the geographical distribution of pollen or closely related vegetation. The biological events contributing to sensitization and the impact of different routes of sensitization are poorly understood and further studies are essential.

The increasing trend in use of *Cannabis* for various purposes may contribute to widespread allergic reactions to *C. sativa*. Based on recent findings, certain individuals may be at risk of serious complications such as severe anaphylaxis. The scientific tools currently available for clinical evaluations are limited and inconsistent as highlighted previously (Tetrault et al. 2007). For improved characterization, additional studies are needed to clearly identify at-risk populations and develop standardized methodologies to develop strong diagnostic techniques for rapid therapeutic interventions. The research may also help in development of reagents that may be used for immunotherapy for atopic individuals in the future.

12.7 Conclusion

Cannabis allergic sensitization is a complex physiological condition with manifestation of diverse clinical symptoms that are likely governed by immunophysiological mechanisms that are currently poorly understood. The identification of putative allergens using serum from reactive subjects has assisted in gaining critical understanding of the underlying mechanism of the disease condition. However, it is also evident that additional research is required for delineation of a role for cannabinoids that form a major component of the plant biomass. Gaining access to plant components and their application in clinical research is a major limitation in developing an understanding of how plant components interact with human physiology; but may change as the legal status of the plant is deliberated upon. This will aid in developing standardized clinical diagnostic tools and knowledge that will assist clinicians and researchers in dealing with a growing health concern.

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