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亞致死劑量益達胺對蜜蜂生存衝擊

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摘 要

益達胺（imidacloprid）與其他新類尼古丁（neonicotinoid）藥劑的使用，對非目標昆蟲、蜜蜂以及其他授粉昆蟲的族群生存造成嚴重的危害。本篇文獻回顧針對半致死以及亞致死劑量（或濃度）益達胺對蜜蜂個體以及族群所造成的影響進行統整。暴露在亞致死劑量（或濃度）益達胺污染環境下的蜂巢，巢內工蜂顯著減少、封蓋率下降、且巢框重量減少；從行為層面，亞致死劑量益達胺會造成工蜂訪花行為改變、造成工蜂嗅覺學習能力受損、減少工蜂之間的溝通行為；生理層面，外勤蜂的代謝力降低；基因層面顯示，工蜂的免疫反應、氧化還原反應、解毒相關基因等表現受到影響。工蜂幼蟲的試驗指出，取食到亞致死劑量（或濃度）益達胺的幼蟲的發育延遲、發育成之工蜂嗅覺學習能力喪失、早熟、且壽命減短；而幼蟲之解毒、醣類代謝、代謝途徑等相關基因表現量會受到影響。蜂后的活動力、代謝率、產卵率、以及存活率都會下降。益達胺對蜜蜂造成多層面、難以估計的負面影響，其使用需要更謹慎的評估，以降低其對生態造成的衝擊。

關鍵詞：蜜蜂、亞致死效應、益達胺、早熟型外勤蜂、差異性表現基因。

前 言

殺蟲劑的使用是為了去除植物上的害蟲，以達到植物與作物保護的目標。然而，這樣的使用卻也使得授粉者受到影響，大量昆蟲授粉者的消失已經造成全球生態系統以及農業產品的嚴重損失 (Biesmeijer *et al.*, 2006; Klein *et al.*, 2007; Kremen *et al.*, 2007; Gill *et al.*, 2012)。昆蟲授粉的價值估計有 2120 億美金，占了農業生產的 9.5% (Gallai *et al.*, 2009)。其中又以蜜蜂對農業以及全球生態系統影響最深，在絕大部分需要昆蟲授粉的作物中扮演最主要的授粉者角色，約佔了昆蟲授粉的 80%（Free, 1970; Kevan, 1999; Delaplane and Mayer, 2000; Klein *et al.*, 2007）。然而，過去二十年來，歐洲、北美洲等地區出現了成蜂突然大量消失的現象，蜂箱周圍卻只有發現少數成蜂的屍體，推測是蜜蜂外出採集後，因某些因素造成無法歸巢，死於野外，此現象稱為蜂群衰竭失調症候群（Colony Collapse Disorder, CCD) (Stokstad, 2007; Brown and Paxton, 2009; Potts *et al.*, 2010)。不同於一般急性死亡現象，蜂群衰竭失調症並沒有在巢附近找到屍體，且巢內僅有少量工蜂、幼蟲與蜂后，推測是外勤蜂群受到各種不利因素影響，導致其外出採集時難以歸巢而死於野外。突如其來之外勤蜂數量驟減，迫使原本應負責巢內工作之內勤蜂提早轉變為早熟型外勤蜂（precocious forager）。因其飛行與覓

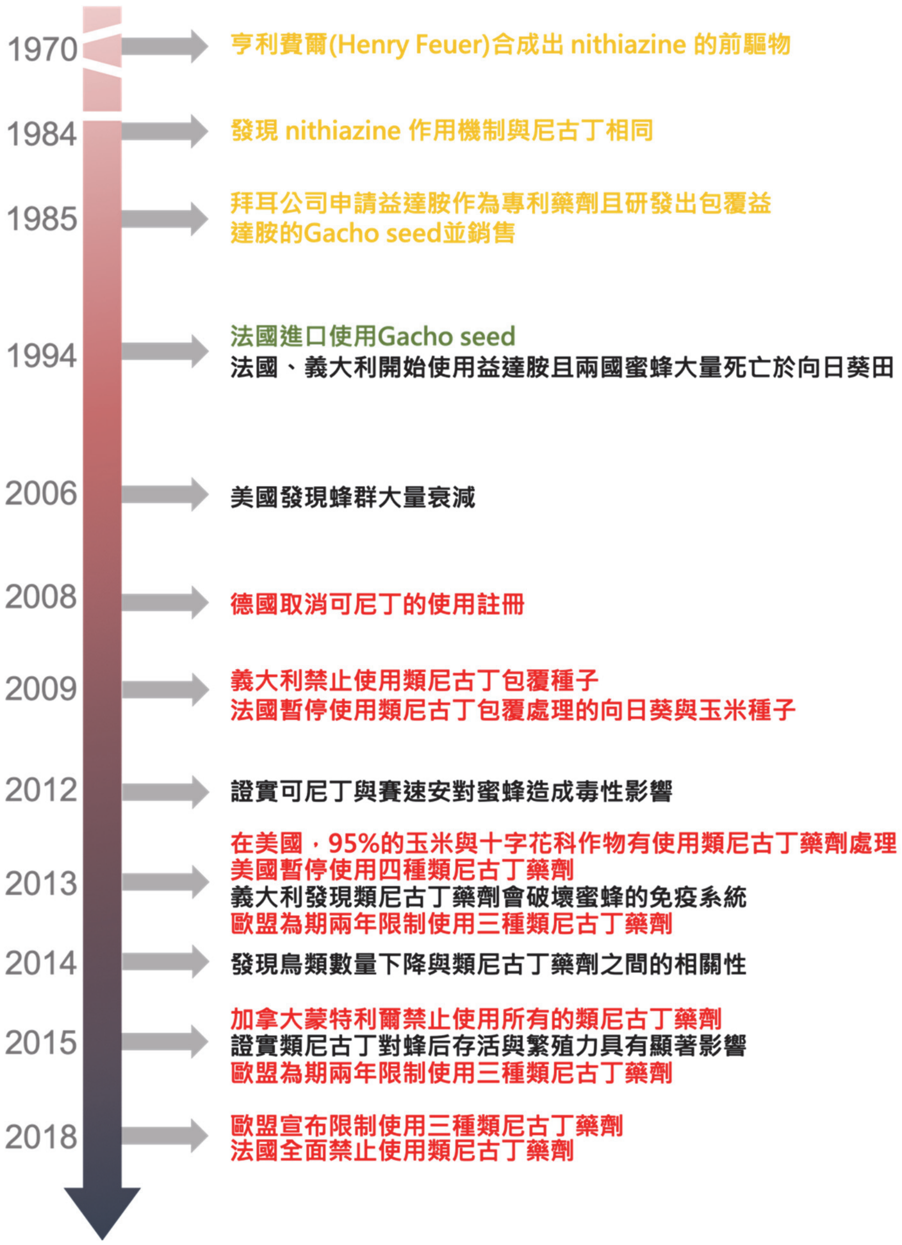
食能力皆遠低於正常之成年外勤蜂，造成早熟型外勤蜂群於外出覓食期間死亡無法返回蜂巢。此一趨勢導致工蜂為覓食而離巢卻消失於田野中，最終造成族群日益衰弱並邁向滅亡 (Schippers *et al.*, 2006; Brown and Paxton, 2009; Potts *et al.*, 2010; Perry *et al*., 2015; Colin *et al*., 2019)。

一般認為 CCD 是由複合因子所導致，如病原體、病毒、寄生蟲、營養失衡、及農業使用之化學藥劑等 (Blanchard *et al.*, 2008; Higes *et al.*, 2009; Maori *et al.*, 2009; Smith *et al.*, 2013; Goulson *et al.*, 2015; Branchiccela *et al.*, 2019)。在可能造成 CCD 的眾多原因之中，蜜蜂的化學中毒最為引起重視。現代農業依賴於化學合成藥劑做農地雜草、害蟲之防治，農地的擴展也讓蜜蜂的採集範圍與農業用地高度重疊，因此暴露於這些化學物質中的機率隨之增加 (Thompson, 2003; Desneux et al., 2007; Goulson *et al.*, 2015; Pisa *et al.*, 2017)。化學藥劑中，以「新類尼古丁類」(neonicotinoid) 殺蟲劑 (例如，益達胺 (imidacloprid)、可尼丁 (clothianidin) 和賽速安 (thiamethoxam) 等) 之大量使用，是目前認為造成蜜蜂消失的最大可能主因 (Henry *et al.*, 2012; Goulson, 2013; Lu *et al.*, 2014; Goñalons and Farina, 2015; Pisa *et al.*, 2021)。新類尼古丁殺蟲劑是尼古丁 (nicotine) 的類似物，為一種神經毒殺蟲劑。1970 年 Henry Feuer 合成 nithiazine 的前驅物 (Feuer and Lawrence, 1969)，在確認此物質之作用機制與尼古丁相同 (Schroeder and Flattum, 1984) 後，Bayer CropScience 即以其為基準，合成研發出第一種新類尼古丁殺蟲劑益達胺，並於 1985 年申請專利，1991 年上市販售，益達胺也成為新類尼古丁藥劑中最為廣泛使用的殺蟲劑 (Kagabu, 2010)。隨著新類尼古丁殺蟲劑的販售與使用，1994 年起不明原因的蜜蜂消失事件開始發生，從歐洲到美國都有不明原因的蜜蜂消失。在眾多科學性的探討研究推論下，發現新類尼古丁殺蟲劑的使用極有可能是造成蜜蜂消失的原因之一，因此德國於 2008 年暫停可尼丁的註冊與使用 (https://www.theguardian.com/environment/2008/may/23/wildlife.endangeredspecies)，歐盟更於 2018 年決定禁止包括益達胺、賽速胺、和可尼丁三種新類尼古丁殺蟲劑之使用 (圖一)。

蜂產品中新類尼古丁藥劑殘留與其對蜜蜂之危害

新類尼古丁藥劑可溶於水，由植物根部吸收並在體內移行，是一系統性殺蟲劑 (systematic pesticides) (Schmuck, 1999; Schmuck *et al.*, 2001)。新類尼古丁類殺蟲劑進入昆蟲體內易與昆蟲神經系統中的菸鹼酸乙醯膽鹼接受器 (nicotinic acetylcholine receptor, nAChR) 結合 (Buckingham *et al.*, 1997; Matsuda *et al.*, 2001)，使神經系統過度興奮，最後造成昆蟲麻痺或衰竭而亡 (Matsuda *et al.*, 2005)。暴露在高劑量的新類尼古丁類殺蟲劑下，蜜蜂會快速死亡，然而暴露在亞致死劑量時，外勤蜂仍能執行勤務，進而將低劑量的農藥帶回蜂巢，隨之導致巢內個體暴露在農藥的污染之下 (Chauzat *et al.*, 2006; Škerl *et al.*, 2009; Codling *et al.*, 2016; Mitchell *et al.*, 2017; Böhme *et al.*, 2018)。

亞致死劑量在本篇文獻回顧泛指低於半致死劑量 (LD_{50}/LC_{50})、不會造成實驗動物明顯死亡的劑量。暴露在此劑量下的實驗動物可能會表現出行為改變、壽命縮短、發育速度、群體數目、繁殖力、性別比例之改變，甚至畸形等行為生理異常現象 (Desneux et al., 2007; de França *et al.*, 2017)，而此劑量的農藥極有可能造成世界性的授粉昆蟲族群下降，這樣的趨勢威脅著由這些授粉者所提供整個生態系的授粉服務及依賴他們而生存的生物多樣性。雖然新類尼古丁藥劑被認為是威脅授粉昆蟲生存的主要原因，但是過去一直缺乏全球性的調查確認是否蜂類正暴露在這些化學物質所污然的環境之下。2017 年 10 月發表在「Science」期刊中的一篇研究，Mitchell *et al.* (2017) 分析了來自世界各地的 198 個蜂蜜樣品，檢測其中包括亞滅培，可尼丁，益達胺，賽果培，賽速安五種農藥的殘留量，發現 75%的樣品中至少含有所檢測的五種農藥中的一種，45%的樣品中含有兩種以上，10%的樣本含有四種或五種。這些結果確認了全世界大部分的蜂類正暴露於新類尼古丁藥劑的環境中。雖然在蜂蜜樣品中所量測到的藥劑濃度 (平均值：1.8 ng/g) 低於危害人體健康的攝取量，但是約一半 (48%) 的蜂蜜樣品裡的藥劑濃度超過 10 ng/g (10 ppb)，而這個濃度的藥劑含量已經被證實是足以對蜜蜂健康與行為造成負面影響 (Yang *et al.,* 2008; Peng and Yang, 2016)。此項研究並未探討蜂蜜品質，但是對蜂類族群以及養蜂人而言卻是個警訊，因為大部分的授粉



圖一　新類尼古丁殺蟲劑與蜜蜂消失事件時間軸。新類尼古丁殺蟲劑從研發、上市、證實對蜜蜂造成毒性影響、到歐盟宣布禁止三種新類尼古丁殺蟲劑使用之時間。黃色：類尼古丁殺蟲劑的研發；綠色：類尼古丁殺蟲劑披衣處理的種子上市販售；黑色：重大不良影響事件；紅色：官方禁令之宣布。

Fig. 1. Timeline of neonicotinoid application and occurrence of colony collapse disorder. Yellow: the investigation and development of neonicotinoid pesticides; Green: neonicotinoid-treated seeds on the market; Black: key events with negative effects (e.g., neonicotinoids confirmed to be toxic to honey bees); Red: announcement of the official ban by the European Union.

昆蟲都暴露在高農藥殘留的環境中。基於此研究結果，新類尼古丁藥劑的使用是無法與蜂群以及生態系的長期維護互相兼容的 (Lawrence *et al.*, 2016; Pisa *et al.*, 2021)。

近年來的研究結果指出，即使是不會直接造成生物立即性死亡之亞致死劑量，新類尼古丁藥劑對訪花蜂類昆蟲（包括馴養的蜜蜂和野蜂）仍會造成明顯有害的影響。低濃度的新類尼古丁藥劑可造成授粉昆蟲在行為、生理及生殖上的影響：不正常的生長發育、降低免疫系統的效能、神經或認知功能上的失調、呼吸及生殖功能的不正常、蜂王壽命的減短、產生的後代數減少、工蜂採集花粉及花蜜的能力下降，以及失去找尋回巢路徑的能力。這些影響讓蜂群變弱，蜂群也失去了抵抗其他逆境因子的能力（例如病原菌、蜂蟹蟎等)(Pettis *et al.*, 2012; Doublet *et al.*, 2014; Lu *et al.*, 2014; Alburaki *et al.*, 2015;

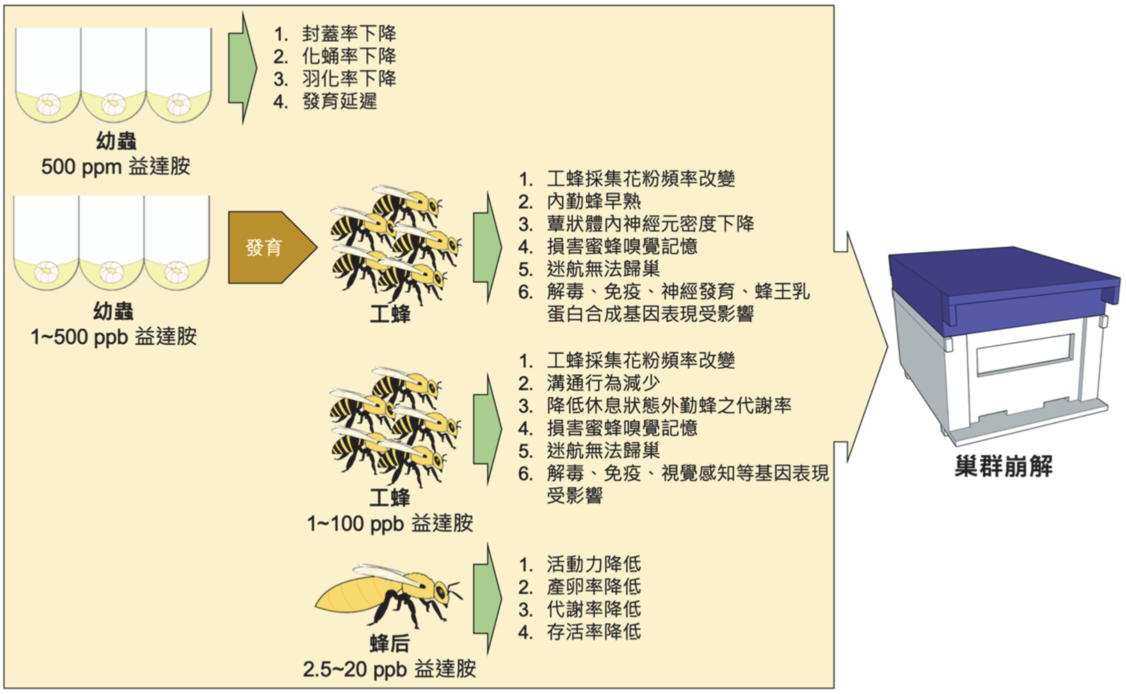
Goulson *et al.*, 2015; Van der Zee *et al.*, 2015; Williams *et al.*, 2015; Morgen and Lundgren, 2016; Sánchez-Bayo *et al.*, 2016; López *et al.*, 2017; Fauser *et al.*, 2017; Pisa *et al.*, 2021)。新類尼古丁當中，益達胺是最為普遍使用的，因此以下文章將以益達胺為主，探討新類尼古丁藥劑對蜜蜂工蜂所造成的影響。

益達胺對蜜蜂的毒性

法國及歐洲各國從 1994 年開始使用益達胺拔衣處理 (seed coating) 的向日葵種子 (高巧種子 Gaucho®) 之後，發現蜜蜂行為異常而無法返巢，最後大量死亡於向日葵田間，導致蜂群的衰弱以及蜂產品產量銳減 (Schmuck *et al.*, 2001; Bonmatin *et al.*, 2005)。此高巧種子的事件使益達胺對蜜蜂所造成的影響開始受到國際間的重視，也開啟了一連串益達胺對於蜜蜂的毒性研究 (Schmuck, 1999; Schmuck *et al.*, 2001; Suchail *et al.*, 2000; 2001; Decourtye *et al.*, 2003; Decourtye and Devillers, 2010)。益達胺對蜜蜂之毒性會因為環境因素和日齡、基因表現等個體差異而有所不同 (Suchail *et al.*, 2000; Laurino *et al.*, 2013; Goñalons and Farina, 2015)，其對蜜蜂工蜂有很高的口服毒性，對單隻蜜蜂的口服半致死劑量 (LD_{50}) 為 3.7~102 ng，而口服半致死濃度 (LC_{50}) 為 140~1,570 μg/kg (Nauen *et al.*, 2001; Schmuck *et al.*, 2001; Suchail *et al.*, 2001; Laurino *et al.*, 2013)，接觸之半致死劑量則為 17.9~242.6 ng (Suchail *et al.*, 2001; Iwasa *et al.*, 2004)。蜜蜂幼蟲對益達胺具有較高的耐受性，口服半致死劑量為 4.17 μg，口服半致死濃度則為 38.84 mg/L，但幼蟲發育仍會受到益達胺影響。當蜂巢中有高劑量的益達胺殘留時，羽化日期會晚一至兩天，且壽命會比正常蜂巢中飼育的蜜蜂短四天，存活率也較低 (Woyciechowski and Moroń, 2009; Wu *et al.*, 2011)；而壽命較短的蜜蜂會有提前轉變成外勤蜂的現象，減少了內勤蜂的比例，對於蜂群大小以及發育能力造成很大的影響 (Tofilski, 2009; Colin *et al.*, 2019)。

亞致死劑量益達胺對蜜蜂行為生理與巢群之影響

半致死劑量 / 濃度 LD_{50} 及 LC_{50} 為殺蟲劑的主要毒性指標，然而根據歐美學者的調查報告，在自然環境中，土壤、花蜜和花粉內所含的益達胺濃度約在 10 μg/kg (10 ppb) 左右，遠低於半致死劑量 (Curé *et al.*, 1999; Schmuck, 1999; Schmuck *et al.,* 2001; Bonmatin *et al.,* 2003; Decourtye *et al.*, 2004)。前人評估亞致死劑量的益達胺對蜜蜂的影響發現，益達胺濃度為 0.5 μg/L 和 5 μg/L 會造成蜜蜂改變採集花粉的頻率和封蓋率下降 (Faucon *et al.*, 2005; Meikle *et al.*, 2016)；1-20 μg/kg 和 0.1-2 ng/bee 的劑量會對蜜蜂採集產生嚴重影響 (Colin and Bonmatin, 2000)；6~100 μg/kg 會改變採集的活動力 (Bortolotti *et al.*, 2003; Decourtye *et al.*, 2003; Colin *et al.*, 2004; Ramirez-Romero *et al.*, 2005)。當蜜蜂取食 50 μg/L 的益達胺蔗糖溶液後，往返巢房與人工餵食器之間的時間就會延長，隨著餵食的益達胺濃度增加，回巢率會降低。由此可知，即使益達胺尚未達到可以直接殺死蜜蜂的程度，但對蜜蜂的採集行為已經造成了顯著的影響 (Yang *et al.*, 2008)。Decourtye *et al.* (2003) 以 12~24 μg/kg 的益達胺溶液餵食蜜蜂，會造成蜜蜂的學習記憶能力受損。蜜蜂攝入濃度為 100 μg/kg 的益達胺後，會造成其移動性下降和溝通行為減少，進而危及到蜜蜂社會性行為 (Medrzycki *et al.*, 2003)。益達胺劑量為 12 ng/bee 會損害蜜蜂的中期嗅覺記憶 (medium-term olfactory memory) (Decourtye *et al.*, 2004)。暴露在劑量 1.5 ng/bee 的益達胺會造成採集活動顯著減少以及增加飛行時間 (Schneider *et al.*, 2012)。取食含有 5 μg/L (5 ppb) 濃度益達胺之外勤蜂在休息狀態的代謝率明顯下降 (Gooley and Gooley, 2020)。巢群暴露在 100 ppb 益達胺下連續六個星期，巢內工蜂顯著減少、封蓋率下降、且巢框重量減少 (Meikle *et al.*, 2016)。蜂群取食含有 5 ppb 益達胺糖水連續 10 天下，所形成的蜂球溫度較控制組高 (William *et al.*, 2018)。暴露在 5 ppb 益達胺下即會造成蜂群的過冬存活率明顯下降，且存活率隨著益達胺的濃度增加而降低 (Dively *et al.*, 2015)。除此之外，益達胺對於蜜蜂腦部發育影響之研究指出，在餵食剛羽化之蜜蜂 8.09 ng 益達胺後，會使 Africanized *A. mellifera* 的蕈狀體細胞核產生染色質濃 (condensed chromatin)，意即細胞死亡。此外，此劑量益達胺更造成肯氏細胞 (Kenyon cell, KC) 產生細胞腫脹 (cell swelling) 的現象 (de Almeida Rossi *et al.,* 2013)。以上這些研究數據顯示，益達胺的亞致死劑量 (或濃度) 即可造成蜜蜂慢性中毒而影響其行為，進而影響蜂群的健康與蜂勢之維持 (Guez *et al.*, 2001; Bonmatin



圖二　不同發育時期蜜蜂暴露在亞致死劑量益達胺下所受到的影響，最終導致巢群崩解。
Fig. 2.　Effects of sublethal dosage of imidacloprid on different developmental stages of honey bees. Imidacloprid exposure eventually results in the collapse of the honey bee colony.

et al., 2005)。除了經由食物攝取之外，蜂巢巢材內的農藥累積也造成了影響，Pareja *et al*. (2011) 的調查顯示，衰弱中的蜂窩 (depopulated beehives) 裡蜂蠟有相對高的益達胺殘留。因此可合理地推測蜂巢中的益達胺會累積於這些物質中，造成蜂群在衰弱前持續暴露在益達胺慢性毒害。而這樣的毒害，除了蜂后、雄蜂、內勤蜂外，幼蟲也持續受到益達胺的慢性危害。

幼蟲對益達胺有較高的耐受性，且需直接餵食幼蟲益達胺 500 ppm 以上的劑量才能影響封蓋率、化蛹率及羽化率，並造成發育遲緩 (Decourtye and Devillers, 2010; Wu *et al*., 2011; Yang *et al*., 2012)、提早離巢等現象 (Colin *et al*., 2019)。低劑量益達胺雖然不會造成幼蟲死亡，但給予幼蟲 0.04 ng/larva (濃度 10 ppb，1 μL 連續餵食四天) 以上的益達胺，羽化後的學習能力就明顯降低，顯示極有可能幼蟲神經細胞的發育受到影響 (Yang *et al*., 2012)。為了釐清幼蟲時期取食到亞致死劑量益達胺對工蜂腦部發育的影響，Peng and Yang (2016) 利用免疫組織學技術，檢驗蜜蜂大腦內的神經發育情形，確認了蜜蜂幼蟲時期餵食亞致死劑量益達胺會使其成蟲腦部蕈狀體內的突觸單元密度 (microglomerular density) 下降，且在益達胺劑量為 0.004 ng/larva 便會造成顯著下降，下降之趨勢會隨著餵食益達胺劑量增加而加劇，直接證實了嗅覺學習能力的下降與蕈狀體中神經連結的異常相關，亦即 1 ppb 的益達胺對蜜蜂的影響從幼蟲期即對神經系統發育造成損害，進而導致神經系統受損 (Peng and Yang, 2016)，造成學習記憶能力不佳。這些學習能力已受損的蜜蜂外出採集時，可能因無法記憶採蜜位置與歸巢路線而不能順利回巢。

蜂后的食物為蜂王乳，蜂王乳為護士蜂 (nurse bee) 所分泌的營養物質，也因此蜂后並不會直接取食到農藥污染之食物。然而，如果護士蜂暴露在亞致死劑量益達胺污染之環境下時，所分泌之蜂王乳中也會檢測到其殘留 (Dively *et al*., 2015)，讓蜂后經由蜂王乳取食到亞致死劑量益達胺。除此之外，蜂蠟內所累積的益達胺，亦是另一個污染源，長時間居住在含有亞致死劑量益達胺之蜂巢內，極有可能造成影響。實驗指出，蜂后暴露在亞致死劑量益達胺下，會降低其產卵率、代謝率、活動力、甚至存活率，且影響隨著劑量上升而更加嚴重 (Williams *et al*., 2015; Wu-Smart and Spivak, 2016; Vergara-Amado *et al*., 2020)。

亞致死劑量益達胺對蜜蜂基因表現的影響

目前已有多篇報導從基因層面探討亞致死劑量

益達胺對工蜂基因表現造成的影響。農藥中毒實驗之中，所檢測的目標基因多與 cytochrome P450 superfamily 的氧化還原反應等解毒相關。結果顯示，工蜂的免疫反應 (immune response)、解毒基因 (detoxification gene) 的氧化還原反應 (oxidation-reduction) 等途徑的相關基因表現受到影響 (Chaimanee *et al.*, 2016; De Smet *et al.*, 2017; Gregorc *et al*, 2018)。而使用次世代定序技術 (NGS, next generation sequencing) 來檢視轉錄組 (transcriptome) 上之改變，發現除了以上途徑之外，光轉導 (phototransduction)、視覺感知 (visual perception)、軀體肌肉發育 (somatic muscle development)、代謝途徑 (metabolic pathways)、行為反應 (behavioral response) 等相關基因表現亦會受到影響 (Wu *et al.,* 2017a; Christen *et al.*, 2018; Li *et al.*, 2019)。

工蜂幼蟲期暴露在亞致死劑量益達胺後所發育之成蟲狀態，也有數篇文獻在基因層面上探討。工蜂幼蟲暴露在 2 μg/L 益達胺下發育後，解毒、細胞外覆結構 (external encapsulating structure)、其他醣類降解 (glycan degradation)、血紅素鍵結 (heme binding) 等相關途徑的基因表現量會上升，表現量下降的基因則與代謝相關途徑 (metabolic pathways)、粒線體組成 (mitochondrial part)、醣代謝/醣異生 (glycolysis/gluconeogenesis) 等相關 (Derecka *et al.*, 2013)；Tesovnik *et al*. (2019) 利用 qPCR 檢測幼蟲期給予 20 ppb 益達胺後免疫系統基因表現狀態，發現 Toll pathway、IMD pathway、JNK pathway 之檢測基因表現量均下降，而抗菌肽 (antimicrobial peptides) 和黑化作用 (melanization) 相關基因的表現狀則會因為齡期不同而有所差異，在紅眼蛹的時期，相關基因表現均為上升，但剛羽化之工蜂相關基因表現量則為下降 (Tesovnik *et al.,* 2019)。Wu *et al*. (2017b) 利用次世代定序技術檢測幼蟲期連續餵食四天每日餵食 1 μL 之濃度 500 ppb 的益達胺 (累積量 2 ng/幼蟲) 對成蟲之影響，發現餵食亞致死劑量益達胺造成 7 日齡工蜂 578 個基因表現有顯著改變，已知所影響的生理功能包括：解毒、免疫 (immunity)、感覺處理 (sensory processing)、神經發育 (neuron development)、代謝 (metabolism) 及粒線體 (mitochondria) 等相關機制的基因。其中蜂王乳蛋白成分合成相關途徑的基因表現大量下降，也證實了亞致死劑量新類尼古丁藥劑對蜂類影響不只限於腦部發育，更影響蜂王漿的合成而影響蜂王漿品質，進而蜂王漿成份之改變會延伸影響巢內其他幼蟲與蜂王的發育，導致蜂巢的衰敗。為了探究亞致死劑量益達胺對於不同齡期工蜂發育的影響，Chen *et al*. (2021) 利用次世代定序技術進行轉錄組研究，探討工蜂幼蟲期取食到 1、10、50 ppb 亞致死濃度益達胺後不同發育齡期轉錄組之表現狀態。結果顯示，所有濃度的益達胺均會影響老熟幼蟲以及不同日齡成蟲基因表現狀態，甚至造成第 14 日齡內勤蜂轉錄組與第 20 日齡外勤蜂相似，且影響程度與益達胺劑量呈正相關，推測暴露在亞致死劑量益達胺下發育之工蜂，極有可能會有提早轉化為外勤蜂且提早離巢，又其嗅覺學習能力、記憶力受損，有極高可能離巢後無法歸巢，導致巢內內勤蜂數量銳減甚至消失。亞致死劑量益達胺對不同齡期蜜蜂之影響簡述如圖二所示。

新類尼古丁藥劑與其他農藥之「雞尾酒效應」

目前為止大部分的研究著重在單一種系統性農藥對蜂類產生的衝擊，但事實上田間與自然環境下農藥殘留的狀況更為複雜，新類尼古丁藥劑與其他農藥同時存在的比例甚高。Mullin *et al*. (2010) 針對北美洲採集了七百多個蜂蠟、花粉、儲粉樣本進行農藥檢驗，其中檢驗出超過一百種農藥與農藥降解物，98.4%的花粉及蜂蠟樣本含有兩種或兩種以上的農藥，80%的樣本含有四種或四種以上的農藥，總計 57%的花粉與蜂蠟含有除蟲菊 (pyrethroid) 與一種或以上的系統性農藥。David *et al*. (2016) 在英國進行花粉取樣，在花粉內檢驗到包括賽速安、可尼丁等新類尼古丁劑藥與白克列 (boscalid) 等殺菌劑 (fungicides) 複合殘留。Daniele *et al*. (2017) 在法國進行蜜蜂、花粉、蜂蠟取樣檢測，亦檢視到複合農藥殘留的現象。而全球蜂蜜採樣針對亞滅培、可尼丁、益達胺、賽果培、賽速安的檢測結果顯示，45% 的蜂蜜樣品含有超過兩種以上的新類尼古丁藥劑殘留，10%的樣品含有四到五種新類尼古丁藥劑殘留 (Mitchell *et al.*, 2017)。Woodcock *et al.* (2017) 在英國、匈牙利及德國放置蜂巢進行田間試驗，發現同樣種類的新類尼古丁藥劑在不同地區會對蜂群造成不同的影響，且他們發現殺菌劑、抗真菌劑等其他藥劑在各個飼養環境內有種類、濃度不一的殘留狀況，這些不同劑量的殘留藥劑似乎是影響各地蜜蜂與野生蜂對新類尼古丁藥劑不同反應的主要因素。目前為止已證實殺菌劑與新類尼古丁藥劑或抗蟎劑共同

存在時會產生雞尾酒效應，讓新類尼古丁藥劑和殺蟎劑對蜜蜂的 LD_{50} / LC_{50} 降低，提高兩類藥劑的致死率 (Johnson *et al.*, 2013; Spurgeon *et al.*, 2016; Sgolastra *et al.*, 2017; Tsvetkov *et al.*, 2017)。抗蟎劑與抑制固醇合成 (sterol biosynthesis inhibitor, SBI) 類的殺菌劑之間的協同效應最為顯著，可能原因為 SBI 類殺菌劑會抑制藥物代謝酵素 cytochrome P450 的解毒活性，降低蜜蜂對藥劑的代謝能力而提高抗蟎劑對蜜蜂的致死率 (Johnson *et al.*, 2013)。目前已知會與新類尼古丁藥劑產生雞尾酒效應的殺菌劑為普克利 (propiconazole) 與白克列，僅普克利為 SBI 類藥劑，因此殺菌劑與新類尼古丁藥劑產生雞尾酒效應的確切作用機制仍待探討，而會與新類尼古丁藥劑產生雞尾酒效應的農藥種類更是急待研究釐清。

結　論

過去二十多年以來，為了探索蜜蜂 CCD 的問題而引發針對益達胺及其他新類尼古丁藥劑的大量研究，結果明確地顯示非標的生物暴露在微量的農藥環境中的確會造成各種傷害，包括急性及慢性的毒害。雖然農藥的使用在農業、倉儲等產業與環境下是不可避免的手段，但益達胺與其他新類尼古丁農藥長期大量的使用以及環境逸散狀態下，造成嚴重且難以遏止的生態危機。因此新類尼古丁農藥的使用需要更謹慎的評估，將其對生物的威脅性降到最低點，才能達到生態維持與人類需求雙贏的狀態。

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Effect of Sublethal Imidacloprid on Survival of Honey Bees (*Apis mellifera*)

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ABSTRACT

Imidacloprid and other neonicotinoids are used to kill target pests and for crop protection. However, they also have severe adverse effects on nontarget insects, honey bees, and other pollinators. This review investigates the effect of sublethal imidacloprid on honey bees (*Apis mellifera*) on the individual and colony levels. Beehives exposed to sublethal imidacloprid exhibited a decreased number of workers, capping rate, and hive weight. Workers exposed to sublethal imidacloprid exhibited changes in foraging behavior, less social interaction, and deficiencies in olfactory learning ability. Their metabolic rate was also affected. Molecular evidence suggested that the expression of immune-response, oxidation-reduction, and detoxification-related functions and pathways of the workers were affected. Larvae exposed to sublethal imidacloprid exhibited a delay in development, impaired olfactory learning ability, precocious foraging behavior, and short lifespans after becoming developed adults. In addition, the expressions of detoxification, glycan metabolism, and metabolic pathways were affected. Queens with imidacloprid exposure exhibited low activity and metabolic, egg-laying, and survival rates. The effects of imidacloprid on honey bees are extensive, harmful, and complex. Further evaluation is required to minimize the damage and negative effects of imidacloprid and other neonicotinoids on the ecosystem.

Key words: honey bees, sublethal effect, imidacloprid, precocious forager, differentially expressed gene